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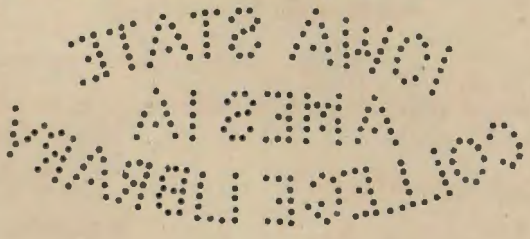

ABSTRACTS OF DOCTORAL THESES[1]

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[1] Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

A STUDY OF *BACTERIUM LINENS*[1]

J. OSCAR ALBERT

From the Department of Dairy Industry, Iowa State College

During the ripening of certain cheeses, a reddish-brown slimy growth commonly develops on the surfaces. The material contains various microorganisms, among which *Bacterium linens* usually is present in relatively large numbers. This organism apparently plays a role in the ripening of certain cheeses, in which it aids in the protein breakdown and flavor development; it also aids in the production of the typical color at the cheese surfaces.

The work herein reported involved: (1) Development of an isolation procedure for *Bact. linens*, (2) studies on its distribution in dairy products and other materials, (3) studies on the general properties of the organism, and (4) preparation of a description of the organism.

The medium developed during the studies and used in making isolations has the following composition:

Ripened cheese	10.0 per cent
Potassium citrate	1.0 per cent
Peptone	1.0 per cent
Sodium chloride	5.0 per cent
Sodium oxalate	0.2 per cent
Agar	1.5 per cent
Water to make	100.0 per cent

pH 7.4

The materials to be examined for *Bact. linens* are smeared on surfaces of plates poured with the agar, either directly or after dispersion in sterile water. The plates are placed under a bell jar or otherwise enclosed, and oxygen is allowed to run into the container slowly for 15 minutes or more, depending on the number of plates to be incubated. The inlet and the outlet tubes are then closed; although 21°C. is a good temperature for incubation, room temperature has been extensively employed and ordinarily gives good results. One week usually is required for good growth and for complete development of color. By using the special cheese agar and following the incubation procedure, *Bact. linens* develops readily. The color production is much more intense and characteristic than when tryptone glucose extract agar or similar media are used with the plates incubated in air and, together with the general colony appearance, permits the detection of the organism with a high degree of accuracy.

The distribution of *Bact. linens* was studied by examining dairy products and various materials from dairy farms and other sources with the general isolation procedure suggested for the organism. A total of

[1] Doctoral thesis number 725, submitted July 9, 1943.

51 samples of foreign type cheeses were investigated and most of them yielded *Bact. linens*. Of 35 samples of cheddar cheese made with raw milk, a rather large percentage contained the organism in relatively small numbers. *Bact. linens* also was found in some of a small number of cheese made from pasteurized milk. The organism was recovered from more than one-half of the samples of milk and cream examined. It was found in most of 59 samples of various kinds of feeds, including corn, oats, barley, and wheat. Certain samples of silage yielded *Bact. linens*. Green plants, hay, and straw yielded it in about one-half of the instances. The organism was recovered rather regularly from water used for watering cows or standing in the barn yards. Mouths of cows yielded *Bact. linens* in several instances when the cows were in the barn, while the organism was not found when the cows were on pasture. *Bact. linens* was obtained from more than one-half of the samples of manure. It was found in the air of various dairy plants, cheese factories, stables, etc. The presence of *Bact. linens* in cheddar cheese made from pasteurized milk may have been due to the organism falling from the air into the milk or the curd. *Bact. linens* was not found in soil.

In litmus milk, *Bact. linens* produced an alkaline reaction and then conspicuous proteolysis. On extended incubation it greatly increased the soluble nitrogen in milk, but different strains varied considerably in the extent of the proteolysis. Amino nitrogen was significantly increased, as were also the fraction soluble in trichloracetic acid and the fractions soluble and insoluble in ethyl alcohol or phosphotungstic acid.

The organism was not lipolytic, but in unsalted butter at 21°C. a putrid condition was produced.

Color production on tryptone glucose extract agar by *Bact. linens* was increased by adding 10 per cent peptone or 5 per cent peptone and 5 per cent casein.

In a medium consisting of 0.3 per cent desiccated yeast extract in water, *Bact. linens* produced volatile acids from various alcohols. Ethyl alcohol yielded practically only acetic acid; propyl alcohol yielded largely propionic acid, and there was evidence of some other acid; butyl alcohol yielded essentially only butyric acid; and amyl alcohol yielded largely valeric acid with a trace of some other acid. In the medium under the conditions used, hexyl and heptyl alcohols yielded very little volatile acid.

In 2 per cent peptone solutions, *Bact. linens* grew at a pH of approximately 6.0 but not at a pH of approximately 5.0. It also grew at a pH of approximately 9.8. In litmus milk in the presence of *Streptococcus lactis*, *Bact. linens* decreased in numbers rather rapidly.

In litmus milk, *Bact. linens* survived for at least 4 months at room temperature. When dried on filter paper it survived for at least 3 months.

The organism grew in the presence of large amounts of sodium chloride; all strains grew in skim milk saturated with it, and some grew in peptone solution saturated with it.

Bact. linens was rather easily destroyed by heat. Only two of the

strains survived 62.8°C. for 5 minutes, which was the shortest exposure used.

All the strains of *Bact. linens* examined were strongly catalase posiive.

A description of the organism was prepared.

SOME APPLICATIONS OF ELECTROMETRIC METHODS TO THE STUDY OF THE COMPONENTS OF STARCH[1]

FRANCIS LESLIE BATES

From the Department of Chemistry, Iowa State College

In recent years investigations in the field of starch chemistry have provided considerable support for the idea that natural starches are composed of two distinct components. Three methods of effecting their separation have been developed (1, 2, 3), and although the mechanism responsible for the separation is different in each procedure, the fractionations that result are quite similar. In each case the starch is separated into two fractions. One gives a deep blue color with iodine, is converted almost completely to maltose by β-amylase, possesses a higher reducing value than does whole starch, and gives crystalline X-ray diffraction patterns upon retrogradation or upon precipitation with alcohol. The other fraction stains purple to red with iodine, is only partially converted by β-amylase, has a low reducing power, retrogrades with difficulty, and produces very poor or amorphous X-ray patterns. Meyer should be given the major share of the credit for perceiving the nature of the fundamental difference between the two components. He has published reviews (4, 5) of the work that have led him to the conclusion that the fraction which stains blue with iodine consists of unbranched starch molecules. The second fraction he identifies with branched chain material, thus limiting to only one component a structure that had been proposed for whole starch by earlier investigators. Meyer calls the unbranched component "amylose" and the branched "amylopectin."

The difference in behavior toward iodine exhibited by the two starch components is easily established in a qualitative manner. The present investigation was initiated by experiments designed to place this behavior on an exact quantitative basis. It was found that the iodine electrode, consisting simply of a bright platinum wire immersed in a solution containing free iodine and iodide ions, provided a very sensitive instrument for measuring changes in iodine activities. Titration of an amylopectin solution with a dilute iodine solution resulted in a steadily increasing iodine activity. In the case of amylose solutions, however, the iodine activity remained almost constant until an amount of iodine had been added equal to about one-fifth the weight of amylose present. The subsequent increase in iodine activity produced an inflection point similar to that obtained in the usual potentiometric titration. The amount of iodine bound by a pure amylose sample under certain well-defined conditions was carefully determined. The great difference in the iodine-binding abilities of amylose and amylopectin was made the basis of an analytical method by which

[1] Doctoral thesis number 729, submitted August 18, 1943.

the amylose content of mixtures of the two components could be determined. Successful in the analysis of known mixtures, it was applied to whole starches and crude starch fractions. The behavior of the two components can best be explained on the basis of a helical configuration of the starch in the complex. This has been confirmed by Rundle and coworkers (6, 7, 8).

Potentiometric iodine titration of starch fractions was used to prove that starch was composed of only two distinctly different components. While the fractionation procedures did not produce complete separation of the two, titration of the fractions showed that one of the methods produced a very good separation and also showed that the crude fractions could be purified effectively. No material was found that had an iodine-binding ability intermediate between those possessed by amylose and amylopectin. The conclusion was drawn that no component existed with structure intermediate between the unbranched chains of the amylose fraction and the highly branched molecules of amylopectin.

The results obtained in the iodine titration of amyloses indicated that longer amylose chains bound iodine at a lower iodine activity than did short ones. It was also found that very short amylodextrins required such high iodine activities that the shortest ones were indistinguishable from amylopectins. This behavior of the amyloses permitted them to be arranged according to their relative molecular weights. It also showed that the amylose fractions obtained from natural starches were quite homogeneous as to chain length. Starch synthesized from glucose-l-phosphate by action of phosphorylase appeared to be a heterogeneous amylose.

The slight affinity of the amylopectins for iodine was also studied, and

TABLE 1
AMYLOSE CONTENTS OF STARCHES

Starch	Amylose, %	Starch	Amylose, %
Waxy corn, defatted	0.5–1	Corn, defatted	26
Tapioca	18	Sago	27
Potato	22	Lily bulb	27, 34
Wheat	24	Pea	29

it was found to vary inversely with the degree of branching. Glycogen, which is more highly branched than amylopectin, had almost no affinity for iodine. Titration of limit dextrins indicated a higher degree of branching than was found in the corresponding amylopectins.

The amylose contents of whole starches determined by the iodine titration procedure showed considerable variation. Table 1 lists a number of starches with their amylose contents.

A number of factors were found to affect the starch-iodine complex formation. They thereby influenced the iodine titration results. The amount of iodine bound by amylose varies inversely with the iodide concentration in the solution presumably because iodide and tri-iodide ions

also enter into the complex with the iodine. Fatty acids and their alkali metal soaps inhibit the formation of the complex and decrease the amount of iodine bound. Since they are present in many whole starches, they must be removed before the iodine titration procedure can be applied. The iodine activity necessary for complex formation with a given amylose varies inversely with the concentration of the amylose itself.

One of the fractionation procedures mentioned above (3) depends apparently on the formation of a crystalline butanol-amylose complex similar in structure to the iodine-amylose complex. It was found that phenol, pyridine, and aniline formed analogous complexes with amylose.

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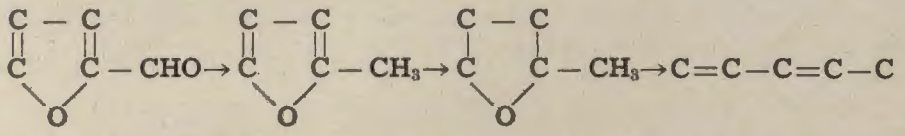
THE PRODUCTION OF RUBBER FROM FURFURAL[1]

LLEWELLYN WILSON BURNETTE

From the Department of Chemistry, Iowa State College

The preparation of piperylene, or 1,3-pentadiene, from furfural has been undertaken with the view of developing a commercially practical process. Although other paths are possible, the following three steps were considered the most direct and feasible. During the course of this



study, a patent[2] was granted which covered essentially the same process, but the claims, particularly of the last two steps, were not substantiated in this laboratory.

Vapor phase catalytic reactions were utilized in the process. For the first two steps a vapor phase hydrogenation apparatus with a recirculation device was employed. This effected the saving of considerable quantities of hydrogen and uncondensed materials. The apparatus could also be employed for the dehydration in the last step.

More than twenty catalysts were studied for the conversion of furfural to methyl furan (sylvan). Only two types were found to be satisfactory. The copper deposited from the decomposition of copper acetate on a carrier such as activated charcoal was found to give yields of the order of 80–85 per cent consistently with one passage of the furfural over the catalyst. The best catalyst tried, however, was copper chromite dispersed on activated charcoal. This has been observed to give a yield of 95 per cent of sylvan in one passage. The optimum temperature observed for these catalysts was around 200°. It is interesting to note that copper chromite in the liquid phase produces furfuryl alcohol (or tetrahydrofurfuryl alcohol according to the conditions used) in quantitative amounts.[3]

Such an efficient conversion of furfural to sylvan applied commercially would make this compound available in large quantities at a low price. With such a stimulus, it should find many important industrial applications. It boils at 64°, has a refractive index of 1.433^{20} and a density of $0.916_{20}{}^{20}$.

The vapor phase hydrogenation of sylvan to tetrahydrosylvan was studied over several types of nickel catalysts. One of the more effective of these was partially activated Raney nickel. After determining that

[1] Doctoral thesis number 728, submitted July, 1943.

[2] H. Guinot (to Les Usines de Melle, Melle, Deux-Sevres, France), U. S. Patent 2,273,484, Feb. 17, 1942.

[3] Calingaert and Edgar, *Ind and Eng. Chem.*, 26: 878–881 (1934).

Raney nickel activated in the usual way led to ring opening and other undesired reactions, Raney nickel activated with only 6–8 per cent of the usual amount of NaOH was used with much better results. The best yields obtained, however, were of the order of 50 per cent. Besides the desired tetrahydrosylvan, pentanone-2 and pentanol-2 were identified in the product in appreciable quantities. Tetrahydrosylvan boils at 78°–79°, has a refractive index of 1.4059^{21} and a density of 0.8534_{15}^{21}.

The dehydration of tetrahydrofurans has been the subject of at least two patents, [2, 4] in which yields of dienes as high as 85 per cent have been claimed. Although the reaction was not exhaustively studied in this laboratory, trials with five different dehydration catalysts have given yields up to only 30 per cent of piperylene. From the amount of water formed, more than twice this much dehydration was indicated, but it is presumed that the yield of the 1,3-fraction was decreased both by the formation of the isomeric 1,4-pentadiene and by decomposition of the desired product.

By carrying out the dehydration at a pressure of 60–85 mm., less decomposition and higher yields were obtained. In the case of the kaolin catalyst studied at a temperature of 400°, the yield of piperylene at atmospheric pressure was 17 per cent. At a lower pressure of about 70 mm. a yield of 30 per cent was obtained.

Piperylene (b.pt. 42°, $n\text{-}1.440^{16}$, d-0.696) was first observed to polymerize to a rubber by Thiele in 1901.[5] Since then, a limited amount of work has been reported in the literature regarding this property. Nothing current has appeared, however, which would serve to compare its properties with the present synthetic rubbers. This research is a necessary precursor to the application of the process on a commercial scale. In this laboratory an emulsion polymerization process similar to that used for butadiene has been used to prepare a satisfactory rubber from piperylene.

The potentially huge quantities of furfural annually available (estimated at 50,000,000 tons) have been emphasized in many instances. Assuming the utilization of but 21 per cent of the annual corn cob crop and mediocre yields in the reactions under discussion, it has been estimated that 100,000 tons of piperylene could be produced at a maximum cost of 38c a pound and at a probable cost much lower than this. This price compares favorably with the current prices of other synthetic rubbers.

In addition to the attractive price, the waste materials utilized in the furfural process are much less critical than the grain and petroleum resources now used. This, even more than cost considerations, should be the deciding factor in its adoption.

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[4] Reppe, Steinhofer, and Hecht (to General Aniline and Film Corp.), U. S. Patent 2,241,792, May 13, 1941.

[5] Thiele, *Annalen*, *319*, 226–30 (1901).

EFFECTS OF CARBON DIOXIDE AND OXYGEN ON ABSORPTION BY ROOTS[1]

HSIEN TSIU CHANG

From the Department of Botany, Iowa State College

The purpose of this study was to evaluate the relative importance of several factors which have been suggested as the causes of reduction of absorption by plant roots in insufficiently aerated media. Wheat, maize, and rice plants were grown in culture solutions under uniform conditions till about 5 to 7 weeks old, then divided into groups receiving different treatments. The treatments included bubbling (1) air, (2) carbon dioxide, or (3) nitrogen through the culture solutions for 10 minutes of each hour; (4) covering the solution with a thin layer of paraffin oil, and (5) controls with no treatment. The period of treatment lasted 36 hours covering approximately 24 hours of daylight and 12 hours of darkness. The amounts of water and nutrient elements absorbed by the plants under different treatments were determined and compared. In the experiment with rice plants, one series of the solutions was adjusted to an acidity of pH 4 by adding 1/10 N sulfuric acid to determine whether the effects of carbon dioxide were due to the increased acidity of the solution. The following results were obtained:

EFFECT OF CARBON DIOXIDE ON WATER ABSORPTION

Bubbling carbon dioxide through the culture solutions reduced water absorption by the three experimental plants by 14 to 50 per cent. In one experiment with wheat, bubbling nitrogen gas through the solution failed to bring about a reduction in water absorption. This indicates that the effect of carbon dioxide on the absorption is not due to its creation of a low oxygen concentration in the solution. The movement of water across membranes is considered to be physical diffusion with little or none

TABLE 1

WATER ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN ML. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

(Each Figure Represents an Average of 6 Replicates)

Crop	Experiment	Ck. (ml.)	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	62.4	112.2	.....	.....	65.1
	II	53.5	106.0	112.9	.....	73.8
Maize	I	33.1	117.5	.....	98.8	85.8
	II	89.2	109.4	.....	99.2	79.8
Rice	Acid+	133.9	99.4	.....	.....	54.8
	Acid−	139.4	111.5	.....	.....	50.2
Average %	.....	.....	100.0	109.3	.....	.....	68.3

[1] Doctoral thesis number 738, submitted December 16, 1943.

of the metabolic effects ascribed to mineral absorption. As a matter of fact water can be absorbed through dead root systems at temporarily increased rates, indicating a resistance factor. Water absorption was increased 9 per cent by aeration, as an average of all experiments. This increase could have been due to the stimulation effect of oxygen or to the removal of carbon dioxide. The failure of sulfuric acid, added to bring the control jars of the cultures to the same pH (4.0) as the CO_2-treated solutions, to significantly reduce water absorption, indicates that the effect of carbon dioxide is not solely through increasing the H-ion concentration of the solution.

EFFECT OF CARBON DIOXIDE ON THE ABSORPTION OF CATIONS AND ANIONS

Tables 2 to 6 inclusive give the effect of different treatments on the absorption of cations and anions. Bubbling carbon dioxide through the solutions reduced significantly the absorption of all nutrient elements tested, by all three experimental plants. Bubbling air through the solution, in general, increased the absorption of the nutrient elements over the control plants, but the increment was much less prominent and consistent than the reduction caused by the carbon dioxide. Bubbling commercial nitrogen through the solutions in a single experiment with wheat did not affect the absorption of cations, but showed some depression on the absorption of phosphate. Covering the solution with a thin layer of paraffin oil caused no great reduction in the absorption of Ca, Mg, and P by maize plants when compared to the controls, but the depressing effect on K and N absorption was significant. Among the various elements, K absorption was most affected by carbon dioxide, loss of K to the solution being obtained in many jars.

Adjusting the acidity of solutions to pH 4.0 produced a reduction in absorption of total salt including NO_3 by rice plants. The absorption, however, was still significantly higher than in plants treated with carbon dioxide.

The results of these experiments support the conclusion that high concentration of carbon dioxide has a specific narcotic effect upon root

TABLE 2

POTASSIUM ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. (Mg.)	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	7.4	256.7	.....	.....	−29.7*
	II	24.0	115.8	106.3	.....	33.7
Maize	I	33.1	129.3	.....	52.9	−23.6*
	II	31.1	166.9	.....	50.8	1.6
Average %	.....	100.0	167.2	.....	.....	−4.5*

* The negative percentages resulted from the release of K by the plants treated with CO_2.

TABLE 3

CALCIUM ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. (Mg.)	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	3.1	45.2	.....	.....	0.6
	II	10.3	95.1	102.9	.....	79.6
Maize	I	8.9	120.2	.....	100.0	57.3
	II	13.1	156.4	.....	119.1	74.0
Average %	.....	100.0	104.2	.....	.....	52.9

TABLE 4

MAGNESIUM ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. Mg.	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	II	7.0	105.7	104.3	.....	55.7
Maize	I	4.1	153.6	.....	100.0	65.8
	II	7.7	115.6	.....	87.0	70.1
Average %	.....	100.0	125.0	.....	.....	63.9

TABLE 5

NITROGEN ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. Mg.	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	I	11.1	161.3	.....	.....	18.9
	II	18.0	97.8	95.0	.....	83.9
Maize	I	10.2	94.1	.....	53.9	36.3
	II	43.9	100.7	.....	94.1	36.7
Rice	Acid+	5.1	107.8	.....	.....	11.8
	Acid−	8.0	143.7	.....	.....	11.2
Average %	.....	100.0	117.6	.....	.....	27.0

TABLE 6

PHOSPHORUS ABSORPTION UNDER DIFFERENT TREATMENTS. CK. IN MG. (EQUALS 100 PER CENT); OTHER TREATMENTS IN PERCENTAGE OF CK.

Crop	Experiment	Ck. Mg.	Aerated %	Nitrogen %	Oiled %	Plus CO_2 %
Wheat	II	2.5	104.0	84.0	.....	64.0
Maize	I	1.5	278.3	.....	66.7	46.7
	II	4.0	207.5	.....	115.0	22.5
Average %	.....	100.0	196.6	.....	.....	44.4

protoplasm which decreases its permeability to water and minerals and apparently deprives the root cells of their ability to absorb ions against a concentration gradient. The important evidences are:

1. Carbon dioxide reduced the absorption of water, a nonelectrolyte, cations, and anions by all three experimental plants.

2. Nitrogen, which also served to sweep out the air from the solutions, failed, in a single experiment with wheat, to reduce the water absorption and cation absorption.

3. Adjusting the pH of control solutions to the value of those treated with carbon dioxide failed to reduce the water absorption. Although it caused a reduction in the absorption of salts, the amounts absorbed were still significantly higher than in plants treated with carbon dioxide.

I. OXIDATIVE DEGRADATION OF CELLULOSE-ACETATE RAYON
II. THERMAL DEGRADATION OF SOME CELLULOSIC TEXTILES BY STEAM[1]

VIRGINIA CHARLOTTE ESTER

From the Department of Chemistry, Iowa State College

I. OXIDATIVE DEGRADATION OF CELLULOSE-ACETATE RAYON

Because references in the literature regarding oxidation of cellulose-acetate rayon are few and contradictory, a study has been made of the oxidizing bleaches, aqueous potassium permanganate, acidic potassium permanganate (0.05 *M* as to sulfuric acid), sodium peroxyborate (0.3 per cent as to soap), neutral calcium hypochlorite, and sodium *N*-chloro-*p*-toluenesulfonamide, on an undyed cellulose-acetate rayon taffeta. Degradation has been followed through changes in acetyl, copper number, wet strength, and weight. These changes have been compared with those observed in unbleached cotton cellulose and regenerated-cellulose rayon when oxidized similarly.

OXIDATION WITH POTASSIUM PERMANGANATE

Acetyl of cellulose-acetate rayon oxidized with either aqueous or acidic potassium permanganate showed an apparent increase, probably because carboxyl groups produced during oxidation reacted with the sodium hydroxide used in saponification for determination of acetyl, and were thus reported as acetyl.

In 4 hours at 40° C., 0.033 *M* aqueous permanganate caused the copper number of cotton cellulose, regenerated-cellulose rayon, and cellulose-acetate rayon to rise 3.56, 6.55, and 0.93, respectively, while in acidic solution the increments were 4.73, 7.69, and greater than 9, respectively. Loss of wet strength in aqueous permanganate under these conditions was 69 per cent for cotton cellulose, complete for regenerated-cellulose rayon, and 43 per cent for cellulose-acetate rayon; in acidic solution the loss was 83 per cent for cotton cellulose and complete loss for regenerated-cellulose rayon and cellulose-acetate rayon. Loss of weight was negligible for all textiles studied in aqueous permanganate, but in acidic bath cotton cellulose lost 3.1 per cent, regenerated-cellulose rayon 9.7 per cent, and cellulose-acetate rayon 45.3 per cent of its weight.

These results indicate that for cotton cellulose and regenerated-cellulose rayon the action of aqueous and acidic potassium permanganate is similar; in most cases effect of temperature is greater than effect of pH. For cellulose-acetate rayon, however, the effect of pH is greater than effect of temperature; this textile, while more resistant to oxidative degradation by aqueous permanganate than cotton cellulose or regenerated-cellulose rayon, is more vigorously attacked in acidic solution.

[1] Doctoral thesis number 737, submitted December 15, 1943.

Oxidation With Sodium Peroxyborate

Acetyl of cellulose-acetate rayon was decreased to a negligible extent by 0.1922 *N* sodium peroxyborate in 8 hours at 40° C. but was reduced by 30 per cent in 2 hours at 100° C. Loss in acetyl was shown to be a linear function of concentration of oxidant, probably because of alkali released during oxidation.

The effect of sodium peroxyborate on unbleached cotton cellulose at 40° C. for 8 hours and at 100° C. for 2 hours is approximately equal and negligible for concentrations up to 0.1922 *N*. Regenerated-cellulose rayon also was attacked equally at both these temperatures but much more considerably than cotton cellulose. Rise in copper number for regenerated-cellulose rayon was 1.84, loss in wet strength 57 per cent, and loss in weight only 0.7 per cent at 0.1922 *N* concentration of oxidant.

The effect of sodium peroxyborate in concentrations up to 0.1922 *N* on cellulose-acetate rayon for 8 hours at 40° C. is negligible. At 100° C. for 2 hours, however, with this concentration, loss in wet strength was approximately 40 per cent, of which only 10 per cent may be ascribed to deacetylation, the remainder to oxidation. Copper number showed progressive linear increase with saponification. Loss in weight was approximately 3 per cent greater than that caused by loss of acetyl, and this, too, was attributed to loss by oxidation.

Oxidation With Calcium Hypochlorite

During treatment with 0.1 *N* neutral calcium hypochlorite, cellulose-acetate rayon was attacked to no noticeable degree until it was oxidized for 4 hours at 40° C. when it lost but 26 per cent of its wet strength, in contrast to cotton cellulose which lost 64 per cent and regenerated-cellulose rayon which was left with no measurable wet strength. In 4 hours at 25° C. cotton cellulose lost 18 per cent and regenerated-cellulose rayon 96 per cent of its wet strength, whereas the wet strength of cellulose-acetate rayon similarly oxidized was unimpaired. Copper number and loss of weight in each case reflected loss in wet strength. No significant change in acetyl of cellulose-acetate rayon was observed in oxidation with calcium hypochlorite.

Oxidation With Sodium-*N*-chloro-*p*-toluenesulfonamide

Neither cotton cellulose nor cellulose-acetate rayon showed any loss in wet strength when oxidized 4 hours at 40° C. in concentrations up to 0.3 *N*, although regenerated-cellulose rayon lost 22 per cent of its wet strength. At 100° C. regenerated-cellulose rayon retained no measurable wet strength even in concentration as low as 0.1 normal. Cotton cellulose retained 77 per cent of its wet strength after 4 hours at 100° C. in 0.3 *N* bath, although cellulose-acetate rayon lost 56 per cent in 0.1 *N* and was disintegrated in 0.2 *N* bath. Copper number and weight of cotton cellulose and regenerated-cellulose reflected these changes. Copper number of cellulose-acetate rayon decreased, although change in acetyl was neg-

ligible, and weight was increased. The increment in weight was thought caused by absorption of the oxidant or its product, *p*-toluenesulfonamide, inasmuch as the textile was yellowed and its depth of color increased with concentration of oxidant.

These results indicate that under mild conditions of oxidation, cellulose-acetate rayon is more resistant than either cotton cellulose or regenerated-cellulose rayon, but that when oxidative action becomes more drastic, it is attacked to a greater extent.

II. THERMAL DEGRADATION OF SOME CELLULOSIC TEXTILES BY STEAM

The action of steam in 1.5 hours at 10, 30, and 60 pounds gauge pressure (115.0°, 134.5°, and 153.0° C., respectively) on unbleached and bleached cotton cellulose, regenerated-cellulose rayon, and cellulose-acetate rayon has been followed by acetyl, copper number, wet strength, and weight. Unbleached cotton lost 75 per cent of its wet strength in 1.5 hours at 153° C. in contrast to bleached cotton cellulose which lost but 47 per cent. Regenerated-cellulose rayon was shown surprisingly resistant to heat, with loss of but 23 per cent of its wet strength. Cellulose-acetate rayon retained only 32 per cent of its wet strengh at 153° C.; this suggested that the loss of strength was brought about by the acetic acid released upon hydrolysis, although loss of acetyl was slight. Loss of weight was negligible for each textile except unbleached cotton cellulose, which lost 4.6 per cent at 153° C. Depth of color of each textile increased as the temperature increased; unbleached cellulose steamed at the highest temperature was of a deep brown color. Starch size provided protection against the action of steam, inasmuch as unbleached and bleached cotton cellulose desized with *Taka-Diastase* were of lower wet strength than when sized.

When increment in copper number was plotted against decrease in wet strength, a line of the general equation $y = mx + b$ resulted within experimental error; m was shown to be different for each textile.

THE PASTEURIZATION OF LIQUID WHOLE EGG[1]

PHILIP ANTHONY GRECO

From the Departments of Animal Husbandry and Bacteriology, Iowa State College

The rapid growth of the liquid whole egg industry during the last 20 years has led to the development of a serious bacteriological problem.

Liquid whole egg is the raw material used for the preparation of frozen and dehydrated egg. It is prepared in egg-breaking plants by breaking the shell-egg, collecting the contents, and thoroughly mixing the product. During the process some precautions are maintained to control, as much as possible, the microbial contamination of the melange. However, in spite of these controls the production of a commercial whole egg containing fewer than 100,000 micro-organisms per gram is comparatively rare. Liquid whole egg containing more than 100,000 micro-organisms per gram deteriorates very rapidly when stored at temperatures above 10° C.

Because of the prevalence of high count egg products in commercial channels, they are constantly under surveillance by public health officials. The presence of pathogenic micro-organisms in such products causing intestinal and other infections in human beings has been suggested from time to time. The possibility of *Salmonella* and *Mycobacterium* infections from egg products is not to be minimized.

These facts led many workers to suggest the possibility of pasteurizing liquid whole egg as a means of improving its keeping qualities and making it "safe." However, it has been reported repeatedly that egg products could not be pasteurized because of the rapid denaturation of the proteins at temperatures which were bactericidal.

This investigation was concerned with studying: the tentative maximum times and temperatures to which egg-melange could be subjected without markedly affecting the proteins, the rate of bacterial destruction by heat of micro-organisms present in egg-melange and the possibility of commercially applying pasteurization to whole egg.

Determinations on the effect of heat on the denaturation of egg-proteins, as measured by the increase in relative viscosity, were made between 56–68°C. Arbitrary standard for tentative allowable denaturation was fixed at 50 per cent increase in relative viscosity. The studies indicated that the rate of denaturation was 250 times more rapid at 68°C. than at 56°C.

Because of its extreme heat resistance, *E. coli* (culture H_1) was used for most of the cell destruction tests. A much less resistant strain of the same organism [P_{19} isolated from egg-melange which had been heated for 1 hour at 136°F. (commercial practice in one plant)] was also studied.

Over 99 per cent cell-destruction of culture H_1 was achieved at the

[1] Doctoral thesis number 734, submitted December 10, 1943.

temperatures studied (56, 59, 62.5 and 66°C.) within the limits imposed by the denaturation of the proteins in the egg-melange. When culture P_{19} was used the period of exposure to produce 99 per cent bacterial destruction at 62.5°C. was less than 1 minute, whereas culture H_1 required 6 minutes.

The age of the eggs used in preparing the melange was shown to have a definite effect on the thermal resistance of bacteria suspended in it.

Bacteria were more easily destroyed by heat in melange having a pH of 7.4–7.6 as compared to that of 6.4–6.8. This effect probably accounts for the differences between the results with fresh and aged eggs. A short growth period (4–5 hours) of culture H_1 in the melange markedly reduced its thermal resistance.

The comparative pasteurizing effect was studied with culture H_1 inoculated simultaneously into egg-melange and raw milk. In every instance it was demonstrated that the bacteria could be more easily destroyed in egg-melange than in milk. The time necessary to produce 99 per cent cell-destruction was 7 minutes in egg-melange and 17.5 minutes in milk. This difference was largely due to pH (pH of milk 6.5, that of egg-melange 7.6). When the pH of egg-melange was adjusted to 6.5, the thermal resistance of the culture in milk and eggs was approximately the same.

The thermal resistance during pasteurization of a strain in a mixture of micro-organisms was the same as when it was tested separately. Over 99 per cent cell-destruction of the mixed organisms was obtained in less than 6 minutes at 62.5°C.

Of the six cultures isolated from liquid whole egg, those of the genus *Actinomyces* were found to be the most resistant.

One commercial trial made with a high-temperature-short-time milk pasteurizer (Creamery Package unit) indicated that egg-melange could be pasteurized effectively (99 per cent destruction) by heating the melange for 32.5 seconds at 67°C. The few micro-organisms which resisted this treatment were identified as members of the species *Leuconostoc citrovorum*, an organism commonly found in milk.

Cake-baking and custard-making tests (using the commercially pasteurized melange) indicated no deleterious effect as a result of this heat treatment.

Other results reported indicate that probably other types of milk pasteurizing equipment could be used successfully if they possess sufficiently sensitive controls.

THE MECHANISM OF FORMATION OF ACETYLMETHYLCARBINOL BY ACTIVE ENZYME PREPARATIONS[1]

NOEL H. GROSS

From the Department of Bacteriology,

Iowa State College

Acetylmethylcarbinol as the precursor to diacetyl is responsible, in part, for the development of desirable flavors in many foods. This fact is especially true of high quality dairy products and bakery goods.

Investigators are not agreed on the origin of acetylmethylcarbinol. Considerable evidence has been presented to support the view that acetaldehyde is an intermediate in the formation of the carbinol from glucose, whereas other investigators have reported the formation of the carbinol from pyruvate.

Silverman and Werkman (1941) were unable to show an increased yield of acetylmethylcarbinol from pyruvic acid when acetaldehyde was added to their cell-free preparation of *Aerobacter*. Green *et al.* (1942) have shown that the addition of acetaldehyde to pyruvate in the presence of a yeast juice gives an increase in the yield of acetylmethylcarbinol.

Active bacterial enzyme preparations were obtained from *Aerobacter aerogenes* by a method developed in this laboratory. Wiggert *et al.* (1940) obtained an active juice from bacteria by mixing a bacterial paste with very finely powdered glass and grinding the mixture in a mortar. Later, a mechanical grinding procedure superseded the earlier method. The bacterial enzyme preparations were very active on glucose or sodium pyruvate when tested on the Barcroft-Warburg respirometer under an atmosphere of nitrogen. The activity was measured by the CO_2 produced and by the amount of acetylmethylcarbinol formed.

The yeast juices were prepared from dried yeast. The yeast was autolyzed and the active principle was precipitated by $(NH_4)_2SO_4$ and redissolved in phosphate solution according to the method of Green *et al.* (1942).

The yeast juices were very active on glucose and sodium pyruvate as shown by manometric determinations. Considerable amounts of acetylmethylcarbinol were formed from pyruvate.

Freezing the yeast juice decreased the rate of formation and the total amount of CO_2 produced from sodium pyruvate.

Increasing the concentration of substrate (sodium pyruvate) increased the amount of CO_2 produced. The amount of acetylmethylcarbinol was increased also. The increases in CO_2 and acetylmethylcarbinol were not proportional to the increase in substrate added.

The addition of acetaldehyde to the yeast juice increased the amount of CO_2 and acetylmethylcarbinol formed.

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[1] Doctoral thesis number 747, submitted June 6, 1944.

The animal juice was obtained from minced pig heart by precipitation with acetic acid and resuspended in phosphate buffer. The activity of this juice, as determined manometrically, was not high because only small amounts of CO_2 were formed from pyruvate. The amount of acetylmethylcarbinol formed, however, was much greater than with the bacterial or yeast juices.

The mechanism of formation of acetylmethylcarbinol was studied using isotopic carbon (C^{13}) as the tracer. Acetaldehyde containing C^{13} in both positions was employed. Fermentations were conducted with the various enzyme preparations using sodium pyruvate and the isotopic acetaldehyde as the substrates.

The acetylmethylcarbinol formed by the bacterial juice contained the normal complement of C^{13}. This finding confirmed the investigations of Silverman and Werkman (1941) that added acetaldehyde did not enter into the carbinol formation by their cell-free juice.

The acetylmethylcarbinol formed in the yeast juice fermentations contained a considerable increase in the C^{13}. The only source of enriched C^{13} was the acetaldehyde. These data indicate that yeast juice can utilize synthetic aldehyde in the formation of the carbinol.

The pig heart juice formed acetylmethylcarbinol from aldehyde alone with no production of CO_2. The addition of pyruvate did not increase the carbinol production.

The isotopic carbon composition of the aceylmethylcarbinol formed by the yeast juice containing the increased C^{13} content was determined. Each carbon group of the carbinol was split out of the molecule and analyzed for C^{13}.

The methyl group, adjacent to the keto carbon, was split off by the iodoform reaction leaving lactic acid. The lactic acid molecule was oxidized to CO_2 and acetaldehyde. The CO_2 contained the carbonyl carbon of the acetylmethylcarbinol molecule. The methyl group was removed from the aldehyde by the iodoform reaction. The formic acid formed by the above reaction contained the carbon from the original carbinol group of the acetylmethylcarbinol.

In other experiments the acetylmethylcarbinol was split into acetic acid and acetaldehyde by the KIO_4 oxidation.

Data obtained indicate that each carbon of the C^{13} acetylmethylcarbinol was enriched with C^{13} but not to the same degree. The carbonyl end of the molecule contained the smaller amount of heavy carbon. The carbinol end contained a greater percentage of heavy carbon than the whole original acetylmethylcarbinol molecule. These data indicate a greater fixation of the synthetic acetaldehyde in the carbinol end of the molecule.

Many dried yeast preparations were investigated to find an active juice, with little success.

An apparatus is described for collecting and weighing the CO_2 directly from the oxidation reaction. This bulb eliminates the barium salt step formerly used with the mass spectrometer analysis.

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MEAT IN NUTRITION. XV. CERTAIN CHARACTERISTICS OF GESTATIONAL PERFORMANCE IN ALBINO RATS FED A DIET CONTAINING DRIED AUTOCLAVED PORK MUSCLE[1]

WILLIAMINA ARMSTRONG HIMWICH

From the Department of Foods and Nutrition, Iowa State College

Reports from the Nutrition Laboratory of the Foods and Nutrition Department at the Iowa State College have shown that feeding rats a supposedly adequate diet containing dried autoclaved pork muscle consistently produces both partial and complete gestational failures. In partial gestational failure, the most striking abnormality is a high mortality of the young during the first 4 days of life; in the complete, an acute disturbance at the time of parturition that results in the death of both mother and feti. The chief purpose of the present study was to establish the syndrome characteristic of each type of gestational failure. In addition, the effect of adding three supplements, lipocaic, fresh liver, and liver extract, to the basal pork diet was investigated.

The 235 animals used in the experiment were divided into three experimental groups, *i.e.*, (1) the control group of rats fed the stock colony diet known as Steenbock V which has never been known to produce complete gestational failure, (2) the group of rats receiving the experimental (basal pork) ration, and (3) the group of animals receiving the experimental ration supplemented by fresh liver, lipocaic, or liver extract. To determine the influence of pregnancy *per se*, pregnant and virgin females were maintained on each diet. The experimental groups of pregnant animals were further subdivided on the basis of the diet of the males used for mating.

The females in the pregnant series were allowed to bear and rear one litter each. They were then killed 21.5 days following the initiation of the second pregnancy. The general physical condition of the animals and the appearance of certain organs were described at the end of the experiment. The liver, kidney, heart, spleen, feti, placentae, and in some cases the pancreas were removed at autopsy. The virgin animals in each group were killed when they had received the diet the same number of days as the pregnant animals in that group.

In the first part of the study the gestational performance of animals in the three experimental groups was evaluated. Data collected on the progression of the first gestation period and the condition and vitality of the first litter were used in this analysis. In addition, data pertaining to the second pregnancy, obtained by observation on the progression of gestation, and the condition of the uterine contents at autopsy were studied. The following observations were made:

1. The second pregnancy was a better measure of the effect of diet upon gestational performance than was the first pregnancy,

[1] Doctoral thesis number 524, submitted June 7, 1939.

2. The feeding of the pork-containing diet resulted in poorer gestational performance than that noted in animals fed the adequate control ration,

3. Gestational failures, both partial and complete, were more numerous in females mated with males also receiving the basal pork-containing diet, than when males from the stock colony were used for mating,

4. Fresh liver was the only supplement added to the basal pork diet that prevented the appearance of the pregnancy disorder, and

5. The feeding of the sample of lipocaic used markedly increased the occurrence of resorptions, as many as 68 per cent of the feti being lost in rats fed 500 mg. of the supplement daily.

The second part of the investigation consisted of a study of the pathological changes associated with complete gestational failure. Such failure occurred in about 35 per cent of the pregnant animals receiving the pork ration. Changes in general physical condition of the animals, gain in body weight during pregnancy, water consumption in pregnancy, fat content of the liver, weight and moisture content of organs, and histology of organs, feti, and placentae were considered.

The general physical condition of the animals both in regard to external appearance and condition of certain visceral organs was rated subjectively. In addition, the rectal temperature of the pregnant animals was taken. The gains made in body weight during gestation were studied, pairing experimental animals with normal females from the stock colony matched in respect to body weight at the initiation of pregnancy and number and weight of feti. The amount of water consumed by the pregnant animals was measured twice daily from the twelfth day of pregnancy until parturition. The average weight and moisture content of the liver, kidney, spleen, heart, and mammae were determined. In addition, analyses were made of the fat content of the liver.[2] Histological sections were prepared of the liver, kidney, heart, spleen, and pancreas, as well as of the feti and placentae. A standard method using Zenker's solution for the fixative and haematoxylin and ethyl eosin as stains was followed in the preparation of the sections.

The feeding of the pork diet to the virgin animals increased the relative quantity of fat in the liver and induced cellular changes in the liver and the kidney. These differences were accentuated by pregnancy. In the control group fed the stock colony diet, the virgin animals were normal and pregnancy produced only slight cellular changes.

Between the apparently healthy pregnant animals fed the various pork-containing diets and the normal control group, the only consistent differences noted were in the liver. The livers from the rats receiving pork muscle were higher in fat and lower in moisture than those of the normal control animals. In addition, an increase in cloudy swelling in the hepatic cells was observed upon histological examination of the sections prepared from livers of pork-fed rats. Some degenerative changes were also noted in the kidneys of these rats.

[2] These data were included through the courtesy of Dr. Ethelwyn Wilcox.

Deviations from normal were marked in the ten animals that died due to the pregnancy disorder. In general, the symptoms may be described as follows:

1. The sick animals made excessive gains in body weight during the last day of pregnancy; the relative moisture contents of the liver, kidney and spleen of these animals as well as changes in water consumption suggested that the large gains in body weight were due to a disturbance in water balance;

2. The liver was yellow in color, large in size, and friable in consistency, the quantity of fat was abnormally high, and the hepatic cells showed marked fat degeneration and infiltration;

3. The kidneys were swollen and gorged with blood;

4. The feti were well-developed, but invariably dead and all showed hemolysis of fetal blood and thrombi in the umbilical veins, and

5. The placentae were anemic.

Finally, an attempt was made to evaluate the significance of the findings. In so doing, similarities between the pregnancy disorder described above, eclampsia in women, and disturbances of gestation reported in rabbits and sheep were indicated. A theory was developed that might explain the train of events observed in the pregnancy disorder; also, the changes found in partial gestational failure were correlated with those observed in complete gestational failure.

The following general conclusions may be drawn from the data:

1. Both the partial and complete gestational failures observed in animals fed the basal pork-containing ration may be prevented by dietary means and hence are due to a lack of some factor or factors in the basal ration,

2. The fundamental disorder of dietary origin is aggravated to a serious level by the imposition of pregnancy,

3. A specific syndrome is characteristic of the pregnancy disorder,

4. The pregnancy disorder occurring in gravid rats fed the basal pork ration is strikingly similar to eclampsia in women and seems typical of a general metabolic disturbance.

THE USE OF SOME AGRICULTURAL PRODUCTS AS RAW MATERIALS IN THE PLASTIC INDUSTRY[1]

JAMES ALVIN JOHNSON

From the Department of Chemical Engineering, Iowa State College

Plastics have developed into a major industry in the last 30 years. The limiting factor of this industry at the present is the cost of raw materials. The plentifulness and cheapness of agricultural products, and particularly certain by-products, make them inviting as possible plastics raw materials. This thesis is a study of the production of low cost plastics from agricultural materials.

FURFURAL-SOYBEAN MEAL PLASTICS

Since it is known that a protein and an aldehyde react to form a condensation product, it would be expected that such a reaction would take place between soybean meal, which is high in vegetable protein, and either formaldehyde or furfural.

In the early studies, furfural, phenol, and ammonia were mixed together in a beaker heated in a bath of boiling water. Soybean meal and lime were added and the pasty mass stirred and heated from 2 to 5 hours, after which it was dried for several days at 60°C. The now brittle material was pulverized and mixed with approximately equal weight of filler (wood flour or asbestos) and 4 per cent hexamethylene tetramine. This mixture was formed into test buttons in a heated mold under hydraulic pressure. In later studies using larger amounts of materials, the cooking was done in a small steel cooker with a power-driven stirrer.

As the result of studies on the effect of such variables as the relative amounts of constituents, cooking time, and method of adding and mixing ingredients, the optimum product was found to be produced as follows: Thirty parts by weight of furfural, 24 parts of phenol, and 4 parts ammonium hydroxide were refluxed for 1 hour. To this were added 36 parts soybean meal and 3 parts lime. The mixture was then heated with constant mixing for 4.5 hours at 110° to 120°C. It was then dried and ground to pass a 60-mesh screen. Forty parts of this ground resin were then mixed with 60 parts of asbestos filler and 6 parts hexamethylene tetramine. The resulting molding powder was molded at 200°C. and 1,800 pounds per square inch for 3.5 minutes, followed by cooling under pressure in the mold for 2 minutes. Test buttons made in this manner withstood a drop test of a 5-pound weight from a height of 24 inches, and had a water absorption of 0.55 per cent in 24 hours, thus comparing favorably with similar types of commercial plastics. When the hexamethylene tetramine was reduced to 2 per cent, the water absorption dropped to 0.14 per cent and the strength to 14 inches. There is also experimental evidence to

[1] Doctoral thesis number 499, submitted December 17, 1938.

show that the substitution of water extracted soybean meal produces a plastic with a lower water absorption than unextracted meal. Plastics using wood flour as a filler had both greater strength and greater moisture absorption than those using asbestos.

Other workers in this laboratory had developed a plastic from corncobs, cresol, and sulfuric acid, which was characterized by low water absorption, good moldability, and excellent appearance. The strength of this plastic was somewhat lower than the soybean product. The two plastic resins were blended together in varying proportions, mixed with filler, and molded. The optimum mixture was found to be 11 parts of the corncob-cresol resin, 33 parts soybean resin, 66 parts asbestos filler, and 6 parts hexamethylene tetramine. Test pieces molded from this mixture failed to break under a drop test of 24 inches (the limit of the testing machine) and had a moisture absorption of 0.33 per cent, thus combining the good characteristics of the two products.

The corncob-cresol plastic used in this mixture was made as follows: One hundred ninety-one grams cresol, 39 grams sulfuric acid (1 part concentrated acid to 1 part water), and 130 grams corncobs were refluxed at 110°C. for 2 hours followed by heating without the condenser to 250°C.

The raw material cost of the furfural-soybean phenol resin is 10 cents per pound. The molding powder with wood flour filler is 7 cents a pound, and with asbestos filler is 6 cents a pound. The material cost for the molding powder composed of the two types of resins with asbestos filler varies from 3.5 to 6 cents a pound depending on the quality of the product produced.

PLASTICS FROM HYDROLYZED AGRICULTURAL BY-PRODUCT MATERIALS

The agricultural by-products furnish the largest supply of organic material in the world. Most of these materials are hydrolyzed by low acid concentrations to produce xylose. The production of xylose from cornstalks was studied as a separate problem, and the hydrolyzed residue remaining from this study was used as a raw material for plastics. The cornstalks were cooked in water at 15 pounds per square inch steam pressure for 2 hours, washed with 0.25 *N* sulfuric acid, cooked at 70 pounds steam pressure with 0.2 *N* sulfuric acid for 2 hours, and washed with water. The hydrolyzed residue was ground to pass a 60-mesh screen and then treated with furfural, aniline, and other reagents. The best product was produced from 67 per cent hydrolyzed cornstalks, 15 per cent furfural, 15 per cent aniline, and 3 per cent lime cooked at 115° for ½ hour. This material had a good appearance, a strength of 23 inches in the drop test machine, and a water absorption of 1.01 per cent. Hydrofurfuramide was substituted for furfural with equally good results. Unhydrolyzed stalks did not produce a good plastic. Excellent products were also made from lignin, furfural, urea, and aniline.

The hydrolyzed cornstalk-furfural-aniline-lime plastic molding pow-

der without filler had a raw material cost of $0.028 per pound. The addidition of filler would reduce this cost.

SUMMARY

Satisfactory plastic molding compounds have been produced from mixtures of soybean meal, furfural, and phenol, and of this material with a molding compound from corncobs, cresol, and sulfuric acid. The raw material cost is about 6 cents a pound.

A good plastic was also produced from hydrolyzed cornstalks, furfural, aniline, and lime at a raw material cost of less than 3 cents a pound.

PATHOGENICITY ON AVENA AND GROWTH RESPONSE OF *PSEUDOMONAS CORONAFACIENS* (ELLIOTT) STAPP[1]

CHARLES HUSTON KINGSOLVER

From the Department of Botany, Iowa State College

In a study conducted in 1940, 1941, and 1942 *Pseudomonas coronafaciens* (Elliott) Stapp, the causal organism of halo blight, was observed to attack species and varieties of Avena from the time the seed coat ruptured until the plant was mature. Symptoms were observed on the coleoptiles, culms, leaf sheaths, leaves, and glumes. The plumule within some infected seed was found to be entirely destroyed, and the radicle remained underdeveloped. In some cases the entire seed, excepting the lemma and palea, was rotted to such an extent that if pressure were applied, a yellowish-white viscous material was extruded. Varying degrees of severity of plumule necrosis were observed ranging from seedlings on which the plumule could not be observed to those in which the above ground parts attained almost full development after emergence. On leaves, the part of the plant most commonly attacked, symptoms appeared first as tiny water-soaked spots 1 mm. or less in diameter which became the centers of lesions showing yellowed, haloed areas, rapidly becoming browned and confluent or of concentrically ringed appearance. The effect of variety of host on symptoms was evident and consisted essentially of variation in color, size and number of lesions, and definiteness of concentrically ringed or confluent appearance. Lesions on leaf sheaths observed were elongate, yellowed, confluent areas commonly lacking the concentrically ringed appearance. Lesions on culms and glumes were observed infrequently.

Numerous isolations were made in 1940, 1941, and 1942 from oat leaf lesions of suspected bacterial origin. The lesions showed considerable variations in size, shape, color, and amount of halo. There was some difference in lesion type between host varieties. Isolates from linear lesions with observable exudate were designated as *Ps. striafaciens* and did not differ in cultural reactions from isolates of *Ps. coronafaciens*. Isolates from *Bromus inermis* identified as *Ps. coronafaciens* var. *atropurpureum* were similar in cultural reactions to isolates of *Ps. coronafaciens*, with the exceptions of the production of fluorescence in beef-peptone broth and their slightly more rapid growth. The isolates from brome were pathogenic on oats.

The isolates from oats and brome grass were observed on and in various selected media to determine whether difference in reaction existed among isolates from different hosts and types of lesions and to provide a basis for comparison with the characteristics of isolates described by previous workers. The characters observed were gross morphology, tempera-

[1] Doctoral thesis number 710, submitted March 15, 1943.

ture relations, gelatin liquefaction, nitrate reduction, hydrogen sulfide production, ammonia production, reaction in litmus milk, "Imvic" reaction, carbohydrate utilization, and starch hydrolysis. Cultural studies of 9 isolates of *Ps. coronafaciens* obtained in 1940, 18 isolates obtained in 1941, and 27 isolates obtained in 1942 showed that the organism present on oats in Iowa in these 3 years was essentially like the causal organism previously described by Elliott.

There was some evidence of the occurrence of cultural strains among the cultures of *Ps. coronafaciens* in carbohydrate utilization—rhamnose, d-galactose, and sucrose; but clearly defined cultural strains could not be established. Isolates of *Ps. coronafaciens* var. *atropurpurem* obtained from *Bromus inermis* and isolates of *Ps. striafaciens* obtained from oat plants were culturally similar to isolates of *Ps. coronafaciens*.

Isolates were tested for pathogenicity by atomizing water suspensions of bacteria on uninjured leaves of seedling oat plants, by hypodermic injection of the suspension into the culms of juvenile plants, and by seed infection. Seed infection was obtained by soaking oat seeds, from which the hulls had been removed, in a water suspension of the bacterial cells, or by placing oat seeds with hulls on in the suspension and holding them under a partial vacuum equal to 22 to 25 inches of mercury for 30 minutes. It was necessary to remove the hulls or place the bacteria under the hulls by use of a partial vacuum before consistent and heavy seed infection could be obtained. Oat varieties inoculated in this manner showed striking increases in pre-emergence killing and in severity of disease on the surviving seedlings. Certain varieties and selections were much more susceptible than others. Varietal response, using this method of inoculation, was in closer agreement with field observations than when plants were sprayed with a suspension of bacteria or inoculated by means of hypodermic injection. The degree of susceptibility of the varieties changed with the conditions under which the test was conducted. The behavior most like the field leaf reaction occurred in the tests at 70°F. At a lower temperature (50°F.) separation of the disease injury into two categories was evident, particularly in the tests with hulled seed. The severity of disease on the leaves agreed in general with the field leaf reaction studies. The second effect, that of pre-emergent killing, seemed to be relatively unrelated to the severity of leaf symptoms. It seems that utilization of this seed infestation method is of real value in evaluation of varietal response in Avena.

The data presented indicate that *Ps. coronafaciens* is a more prevalent and destructive pathogen on oats in Iowa than formerly was realized. It is evident that seedling injury and killing may play a considerable part in the reduction of oat stands and in providing sources of inoculum for subsequent spread. The range of leaf symptoms studied allows the inclusions of several types of leaf injury heretofore not definitely attributed to this organism.

Studies of field reaction of varieties and selections of oats in Iowa revealed striking and consistent differences in susceptibility to *Ps. corona-*

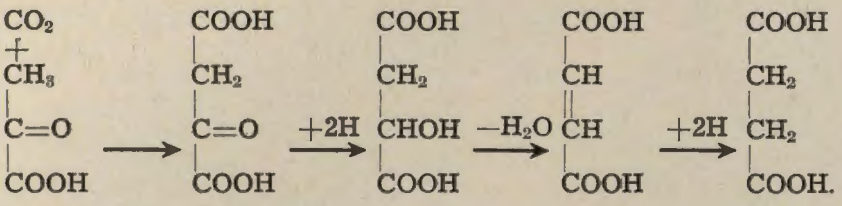
faciens in the 3 years of study. Such examination showed adequate sources of field resistance. Boone, Marion, Hancock, Erban, Anthony, Mutica Ukraina, Gopher, and Landhafer, and selections from crosses involving them as parents, were in general susceptible to halo blight. Victoria x Richland selections were for the most part intermediate in susceptibility although certain selections, such as Boone, were highly susceptible under field conditions. Selections involving Bond as one parent were, in most cases, comparatively free from halo blight. D-69 x Bond selections, as a group, were outstanding for resistance.

PYRUVATE DISSIMILATION BY BACTERIAL ENZYME PREPARATIONS [1]

GEORGE KALNITSKY

From the Department of Bacteriology, Iowa State College

Pyruvate is one of the most important intermediary substances formed during metabolism. It participates in a variety of reactions and is apparently the cardinal intermediary of carbohydrate, fat, and protein metabolism. Wood and Werkman (1940) postulated the carboxylation of pyruvic acid to oxalacetic acid and the conversion of the latter to malic, fumaric, and succinic acids:



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This series of reactions was proposed as the mechanism of heterotrophic utilization of carbon dioxide. The use of the isotopes of carbon has confirmed the occurrence and extended our knowledge of this reaction (*cf.* Werkman and Wood, 1942). In order to further elucidate the mechanism and possible physiological function of the fixation reaction, attempts have been made to utilize another tool, namely a cell-free enzyme preparation.

Bacterial enzyme preparations were obtained by grinding the cells with powdered pyrex glass, according to the method of Wiggert *et al.* (1942), and as further developed by workers in this laboratory. By varying the medium and conditions of growth, cells of *Escherichia coli* were obtained which, on being ground, yielded an enzyme preparation that was quite active anaerobically on pyruvate.

The optimal pH for the dissimilation of pyruvate by the enzyme preparation is 6.77 to 6.80. The preparation can be easily inactivated by heating at 55° to 57° for 5 minutes, and can be conveniently reduced to a powder by freezing and drying *in vacuo* with no immediate loss in activity. The enzymes formic dehydrogenase and hydrogenase are also present, and retain most of their activity even after several months in the dried state.

By subjecting the enzyme preparation to dialysis, it was determined that phosphate, manganese, cocarboxylase, protein, and an unknown substance are necessary for the action of the enzyme system responsible for the anaerobic dissimilation of pyruvic acid to acetic and formic acids.

The main path of pyruvate dissimilation by this preparation is that

[1] Doctoral thesis number 732, submitted November 8, 1943.

leading to acetic and formic acids, but small amounts of carbon dioxide and lactic and succinic acids are also formed.

In manometric experiments small amounts of carbon dioxide are fixed by the enzyme preparation with pyruvate and bicarbonate as substrates. In the presence of $NaHC^{13}O_3$, the fixed carbon dioxide was traced to the carboxyl groups of the succinic and lactic acids formed. Very little excess C^{13} was located in the formic acid, indicating that in the absence of hydrogenlyase, formic acid is not formed by a reduction of CO_2 as such but arises from the carboxyl group of pyruvic acid.

Carbon dioxide fixation is not the only mechanism of succinic acid formation by this enzyme system. On addition of $CH^3 \cdot C^{13}OOH$, succinic acid was isolated containing excess C^{13} exclusively in the carboxyl groups. Therefore, condensation of acetic acid, or its derivative with a 2-carbon or 3-carbon molecule, is another mechanism for the formation of succinic acid.

Evidence supporting the scheme for heterotrophic carbon dioxide utilization (1) has been presented by other workers. Krebs and Eggleston (1941) showed that succinate was formed from pyruvate, oxalacetate, malate, and fumarate by the propionic acid bacteria. Other investigators (Wood *et al.*, 1940, 1941, 1942; Nishina *et al.* ,1941), using the isotopes of carbon, located the fixed carbon dioxide in the carboxyl groups of the succinic fumaric and malic acids formed. Oxalacetate is the prime intermediate in the fixation reaction, but because of the instability and rapid dissimilation of oxalacetate, its direct formation from pyruvate and carbon dioxide has proved difficult. Krampitz *et al.* (1943), using an acetone preparation of *Micrococcus lysodeikticus*, could not demonstrate the formation of oxalacetate *via* direct carboxylation of pyruvate. However, employing the acetone preparation and the heavy carbon isotope, they did demonstrate that during decarboxylation of oxalacetate and carbon dioxide, some carboxylation occurred. This was the first direct evidence that oxalacetic acid or its derivative was a component of the fixation reaction, and that this reaction was reversible.

The enzyme preparation obtained from *E. coli* exhibits strong activity with fumarate and oxalacetate as acceptors of gaseous hydrogen. Oxalacetate is also rapidly decarboxylated. Manganese is necessary for the decarboxylation of oxalacetate, whereas cocarboxylase and inorganic phosphate are not. The specific protein nature of the enzyme concerned in this reaction was demonstrated.

The enzyme forms oxalacetate or a compound closely related to it from fumarate and malate and in smaller amounts from succinate, aerobically, thus demonstrating the reversibility of the reactions postulated above. No oxalacetate is formed from fumarate anaerobically. During the decarboxylation of oxalacetate to pyruvate and carbon dioxide by the enzyme in the presence of $NaHC^{13}O_3$, a carboxylation of pyruvate takes place, and an excess of C^{13} was located in the carboxyl group adjacent to the methylene carbon of the residual oxalacetate.

In attempts to demonstrate the carboxylation of pyruvate, no oxalacetate was detected under optimal conditions for carbon dioxide fixation

and succinic acid formation by the juice under an atmosphere of 10 per cent CO_2 in H_2. Under the same conditions with nitrogen substituted for hydrogen, definite tests were obtained for the formation of small amounts of oxalacetate or a compound very closely resembling it from pyruvate and carbon dioxide. The amounts of "oxalacetate" formed, although small, vary with the concentration of enzyme, pyruvate, and carbon dioxide.

Possible sources of energy for the carboxylation reaction are discussed.

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THE CONVERSION OF FERMENTATION PRODUCTS TO ELASTOMER INTERMEDIATES[1]

JOHN J. KOLFENBACH

From the Department of Chemistry, Iowa State College

INTRODUCTION

A fermentation product, ethyl alcohol, has long been considered the most useful single organic compound, because of the great multitude of its applications in the chemical industry. Ethyl alcohol has recently achieved further prominence by reason of the success encountered in converting the alcohol into butadiene-1,3, the "building block" of synthetic rubber. Many other valuable chemicals besides ethyl alcohol may be produced by fermentation, and the purpose of the work upon which this thesis was based was to investigate the possibility of extending the use of fermentation products, other than ethyl alcohol, for the formation of chemicals for the elastomer industry.

EXPERIMENTAL

BUTADIENE

The production of butadiene by dehydration of 2,3-butylene glycol was first investigated. A series of runs totaling almost 200 was made using a great variety of catalysts and varied reaction conditions, but yields of butadiene never exceeded 10 per cent of theoretical. The predominant reaction product in most of these runs was methyl ethyl ketone. Glycols generally yield carbonyl compounds upon dehydration, according to Ipatieff (1936), and 2,3-butylene glycol proved to be no exception to this rule.

Various esters of 2,3-butylene glycol give rise to butadiene upon pyrolysis. In particular the diacetate of 2,3-butylene glycol has been converted to butadiene in excellent yield on the pilot plant scale at the Northern Regional Research Laboratory. The possibility of converting other derivatives of butylene glycol, the monomethyl ether and the inner carbonate, to butadiene was next investigated.

The monomethyl ether of butylene glycol was prepared according to the directions of Chappell (1935). The inner carbonate had not previously been described. It was prepared by the action of phosgene upon 2,3-butylene glycol, and a continuous counter-current method of reaction was devised. Yields of 75 per cent of theoretical were realized. The compound was found to have the following physical properties: b.p. at 740 mm.=240° C.; d^{25}_4=1.128; n^{25}_D=1.4228; molecular weight in phenanthrene = 116.

	Calc.	Found
Percentage C	51.7	51.5
" H	6.96	7.10

[1] Doctoral thesis number 745, submitted June 5, 1944.

The compound is colorless, insoluble in water, very faint in odor, and a solvent for cellulose esters, all characteristics which suggest its possible utilization as a plasticizer in the plastics and elastomer industry.

Yields of butadiene from both the ether and the inner carbonate of 2,3-butylene glycol were poor. Yields of 13 per cent of theoretical were realized from the monomethyl ether of the glycol, and yields of only 1 per cent were realized from the carbonate. Evidently the inner carbonate, which is a heterocyclic compound, is not as readily pyrolyzed as are the ordinary dialkyl carbonates, which Ritchie (1935) pyrolyzed to unsaturated hydrocarbons.

Methyl, vinyl ketone

The production of methyl vinyl ketone, a material which produces excellent copolymers with butadiene, was investigated. The following chemicals were tried as starting materials for the preparation of methyl vinyl ketone: (1) 2,3-butylene glycol, (2) methylvinylcarbinol, and (3) acetylmethylcarbinol. A method of analysis for methyl vinyl ketone in mixtures with the various impurities present was developed. The analysis employed the polarographic method and was based on the fact that methyl vinyl ketone is reducible at the dropping mercury electrode, whereas the impurities present are not reducible and do not affect the reduction of the methyl vinyl ketone.

The 2,3-butylene glycol proved to be an unsatisfactory source of methyl vinyl ketone. Yields of 5 per cent of theoretical were realized by the simultaneous catalytic dehydration and oxidation of the glycol using a catalyst composed of NaH_2PO_4 on asbestos.

Methyl vinyl ketone yields of 65 per cent of theoretical were produced by the catalytic oxidation of methylvinylcarbinol, using a catalyst composed of zinc oxide (100 g.) and cupric oxide (30 g.). The individual components of the catalyst were inactive but when combined formed a catalyst active at a temperature of 250°C., which is quite low for the catalytic oxidation of unsaturated alcohols. Cuprous oxide was as effective as cupric oxide but was not easily maintained in that particular state of oxidation. Catalytic dehydrogenation of methylvinylcarbinol produced mixtures of methyl vinyl ketone and methyl ethyl ketone, the former being produced in yields of about 30 per cent of theoretical.

The highest yields of methyl vinyl ketone from acetylmethylcarbinol were 13 per cent of theoretical. In general, alcohols having a negative group adjacent to the hydroxyl group dehydrate to unsaturated compounds with difficulty, and acethylmethylcarbinol is such an alcohol. Furthermore, the acetylmethylcarbinol has a great tendency to reduce components of the catalyst, and components such as WO_3, W_2O_5, and $NaHSO_4$ were found to be unsuitable because of this reason.

Methyl acrylate

Methyl acrylate has received considerable attention for production of plastics, and Ziegler (1938) stated that it could be used to form copoly-

mers with butadiene. In recent years, a process for the production of methyl acrylate from lactic acid, a fermentation product, has been developed. This process involves esterification of methyl lactate with acetic acid and pyrolysis of the α-acetoxypropionate at a temperature of about 500° C., a procedure devised by Burns, Jones, and Ritchie (1935) and studied more recently by the staff of the Eastern Regional Research Laboratory. The possible esterification with phosgene instead of acetic acid and pyrolysis of the carbonate instead of the acetate was investigated. Results were encouraging in that a solid polymer could be produced at a relatively low temperature but were discouraging in that the monomer could not be isolated.

Diacetyl

Diacetyl has not been utilized to any great extent in elastomers but is capable of forming polymers with dialdehydes. The production of diacetyl by catalytic oxidation of 2,3-butylene glycol was investigated, and an analytical method employing the polarographic technique was developed. Yields of diacetyl corresponding to 40 per cent of theoretical were attained using a catalyst composed of zinc oxide (100 g.) and cupric oxide (30 g.). Air was used to effect the oxidation and the amounts of air required corresponded to only 1 mol of oxygen per 4 mols of hydroxyl groups.

The utilization of diacetyl for production of polymers was also investigated, and it was demonstrated that diacetyl would condense with either formaldehyde or glyoxal at a pH of 8.0. With formaldehyde a viscous liquid, very probably a mixture of polyhydric diketo compounds was formed, and with glyoxal a solid polymer, suitable for a varnish material, was produced.

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THE FERMENTATIVE PRODUCTION OF GLYCEROL[1]

THOMAS M. LEES

From the Department of Chemistry, Iowa State College

INTRODUCTION

The formation of glycerol during the course of an alcoholic fermentation has been known since the time of Pasteur (1858), but it was not until the period of the first World War that attempts were made to alter the course of the fermentation so as to produce greater quantities of glycerol. Neuberg and his co-workers (1917, 1919) in their investigations on the mechanism of the alcoholic fermentation of sugars by yeast found that acetaldehyde was one of the intermediates in this process. If the acetaldehyde was prevented from being reduced to ethyl alcohol by the addition of sodium sulfite to the medium, glycerol appeared in the medium in quantities equivalent to the amount of aldehyde bound by the sulfite. On the basis of this work the following equation was developed,

(1) $C_6H_{12}O_6 \rightarrow C_3H_8O_3 + CH_3CHO + CO_2$.

As can be seen from this equation, 1 mol of acetaldehyde and 1 mol of glycerol are formed for each mol of hexose fermented.

Connstein and Lüdecke (1921) in Germany and Cocking and Lilly (1922) in England developed processes for the production of glycerol by fermentation based on equation (1). The German process was actually in operation during the last war. The major difficulty with the process appeared not to be in the fermentation itself, but in the recovery of the glycerol from the fermented material.

Neuberg had also shown that in alkaline solution the acetaldehyde underwent a dismutation to produce ethyl alcohol and acetic acid. The overall fermentation reaction was represented as proceeding according to the following equation,

(2) $2\ C_6H_{12}O_6 + H_2O \rightarrow C_3H_8O_3 + C_2H_5OH + CH_3COOH + 2\ CO_2$.

In the United States Eoff (1918) obtained a patent covering the production of glycerol by fermenting a sugar solution in the presence of sodium carbonate. In neither the sulfite nor the alkaline methods for the production of glycerol by fermentation did the yields of glycerol reach the theoretical yields demanded by the equations developed by Neuberg. This failure was attributed to the fact that the normal alcoholic fermentation proceeded simultaneously with the modified fermentations.

There have been numerous other patents and reports in the literature concerning the production of glycerol by fermentation, but most of the improvements have only been modifications of the processes described previously. Hickey (1941) suggested the use of the relatively insoluble magnesium sulfite in place of the more soluble sodium sulfite. The use of the slightly soluble sulfite would simplify the recovery of the glycerol

[1] Doctoral thesis number 746, submitted June 5, 1944.

since most of the sulfite could be readily removed after the fermentation was completed.

With few exceptions, no reports were found in the literature on the use of starchy materials, converted to fermentable sugars, as substrates for the fermentative production of glycerol. Most of the previous work has involved the use of purified sugars, such as sucrose or dextrose, or molasses. In this thesis a study was made of the possibility of using starchy materials as sources of fermentable sugars for the production of glycerol by the sulfite processes.

EXPERIMENTAL

Since the sulfite process for the production of glycerol by fermentation was the only one studied in this work, the reaction between acetaldehyde and the sodium or magnesium bisulfite during fermentation furnished an indirect but rapid method for the determination of the amount of glycerol formed during the fermentation. The method is a modification of the one developed by Tomoda (1929). By iodine titration the amount of free sulfite is determined in a slightly acid solution, and the titration is continued in alkaline solution to determine the amount of sulfite that was bound by the acetaldehyde formed during the fermentation. The acetaldehyde-bisulphite complex is almost completely dissociated in alkaline solution. The amount of sulfite determined in the second part of the titration is equivalent to the amount of acetaldehyde formed which in turn is equivalent to the amount of glycerol present according to equation (1).

Both magnesium sulfite and sodium sulfite were used in this work, and for fermentable substrates enzyme-converted starchy materials, acid-hydrolyzed starchy materials, and purified sugars were employed. It was found that enzyme-converted starchy materials were not satisfactory for the fermentative production of glycerol. Maltose, as the pure sugar, was found to be fermented very slowly by yeast in the presence of sulfites, and since maltose is the chief sugar produced by the action of diastase on starch, the poor fermentations of the enzyme-converted starchy materials were readily understandable. In order to ferment maltose with yeast in the presence of sulfite, the medium must have a pH of 6.9 to 7.1, and suitable nutrients must be present. A 5 per cent maltose solution containing 4 per cent sodium sulfite required 15 days to reach completion, the yield being about 20 per cent glycerol on the initial sugar. A dextrose fermentation under similar conditions was completed in 3 days with a comparable yield of glycerol.

The acid-hydrolysis of corn starch or dry-milled corn products furnished solutions which fermented satisfactorily in the presence of sulfites. By using magnesium sulfite the highest yields of glycerol fell in the range from 22 to 24 per cent glycerol on dextrose, agreeing with the yields obtained by Hickey (1941). With sodium sulfite, yields up to 30 per cent glycerol were obtained, but for yields above 25 per cent high concentrations of sulfite and excessively large yeast inocula were necessary.

The effect of the concentration of sulfite, the concentration of sugar (dextrose), and of the amounts of yeast used for inocula on the yields of glycerol were studied, and all of these factors were found to be interrelated. In several fermentations the pH changes during the course of the fermentation were observed by means of a Cameron automatic pH recorder. The strain of yeast used seemed to have little effect on the yields of glycerol when dextrose or sucrose was the sugar being fermented, but a strain of *Saccharomyces ellipsoideus* apeared to be the best for the fermentation of maltose. It was also found that the temperature and the surface-volume ratio of the fermenting medium affected the yields of glycerol somewhat. An increase of 7° C. in the incubation temperature increased the yield of glycerol by 0.6 per cent. Surface-volume ratios greater than 0.2, a condition usually found only in very small scale fermentations, apparently decreased the yields of glycerol. In general it may be said that the factors which influence the rate of an enzymatic reaction will influence the amounts of glycerol formed by fermentation.

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CUPROUS OXIDE AS A CATALYST: THE EFFECT OF VARYING THE PROPORTIONS OF PROMOTER AND STABILIZER[1]

PHILIP ANDREW LEFRANCOIS

From the Department of Chemistry, Iowa State College

The hydrogenation of furfural to furfuryl alcohol in the liquid phase, using promoted cuprous oxide catalysts, was first reported by Menzel (1) in 1936. Of the promoters investigated, calcium oxide, mechanically added to the Cu_2O, was found to be the best. Stewart (2) extended the use of the cuprous oxide-calcium catalyst to the hydrogenation of acetophenone. He found the mechanical addition of vanadium tetroxide to the catalyst mixture was necessary in order to stabilize the cuprous oxide against reduction to inactive copper. The V_2O_4 also promoted the Cu_2O-CaO catalyst for the hydrogenation of furfural. The adsorptive capacities of promoted cuprous oxide catalysts and their catalytic activity have been investigated by Stanerson (3). The following catalysts, arranged according to decreasing ability, were active in adsorbing hydrogen at 57° C. or higher: Cu_2O-BaO, Cu_2O-V_2O_4-CaO, and Cu_2O-CaO. Cuprous oxide promoted with SrO or MgO adsorbed little or no hydrogen. The Cu_2O-V_2O_4-CaO mixture was considered the best catalyst from the standpoint of rate of reaction, temperature at the start of reaction, adsorptive capacity, and stability against reduction. By determining the optimum proportions of these three ingredients it was hoped to extend the scope of the catalyst. The influence of alkaline earth oxides on the Cu_2O-V_2O_4 mixture was investigated by hydrogenating acetophenone and furfural.

EXPERIMENTAL

A Parr hydrogenation apparatus, Model B3B, of 0.5 liter capacity was used. The weighed quantities of Cu_2O and the promoters, mechanically ground, were inserted into the bomb along with 0.5 mole of hydrogen acceptor. Hydrogen was admitted to an initial pressure of 1,000 lbs./ sq. in. The heater and oscillator were started, and time, temperature, and pressure readings were recorded every 5 minutes. When the temperature of the bomb reached 200° C. the heater was disconnected, and readings continued until the temperature fell to 150°C. at which time the bomb was immediately cooled to room temperature. The pressure readings were converted to absolute pressure at 0°C. in order to have comparable basis for hydrogen consumed. Graphs were constructed for each run plotting decrease in pressure against time, which gave the desired comparison of the rate of hydrogenation. The rate of reaction, arbitrarily defined as the time required for the consumption of 0.45 mole of hydrogen,

[1] Doctoral thesis number 751, submitted June 7, 1944.

the temperature at the start of the reaction, and the total moles of hydrogen adsorbed gave additional comparisons of catalytic activity.

The Cu_2O, V_2O_4, and Cr_2O_3 were prepared according to the directions of Stewart. The oxides of Mg, Ca, Sr, and Ba were used as promoters. Adkins' (4) copper-chromium oxide catalyst was used for comparisons.

RESULTS

A. Calibration of the bomb was found to be necessary for obtaining the actual moles of hydrogen consumed by the hydrogen acceptor. Acetone was hydrogenated quantitatively to isopropyl alcohol. It was found that 0.055 mole of hydrogen was retained by the contents of the bomb over and above that used for complete hydrogenation.

B. Analysis of a carefully dried sample of the Cu_2O catalyst gave approximately 4 per cent water, 94 per cent Cu_2O, and 2 per cent copper as metal. The V_2O_4 was found to be hydrated.

C. Increasing the amount of V_2O_4 in the catalyst mixture of Cu_2O-V_2O_4-CaO lowered the temperature at which reaction started and increased the rate of reaction when furfural was used, but lowered the rate of hydrogenation of acetophenone. Amounts of V_2O_4 greater than half the amount of Cu_2O produced little improvement and are not necessary. The V_2O_4 stabilized the Cu_2O-CaO catalyst in the hydrogenation of acetophenone.

D. Increasing the amount of CaO in the catalyst mixture of Cu_2O-V_2O_4-CaO had a more pronounced effect than increasing the amount of V_2O_4. Increase in the amount of CaO lowered the temperature at the start of the reaction, increased the rate of reaction, and produced a higher yield of hydrogenated product in a shorter time interval when either furfural or acetophenone was used. Furfural containing water reacted more slowly than dry furfural when Cu_2O-V_2O_4-CaO was used, but the activity of the catalyst was decreased and not destroyed.

E. The catalyst Cu_2O-Cr_2O_3-CaO was slightly more active than Cu_2O-V_2O_4-CaO in the hydrogenation of furfural but less active in the hydrogenation of acetophenone.

F. Copper-chromium oxide catalyst of Adkins was less active than Cu_2O-V_2O_4-CaO in the hydrogenation of furfural but more active in the hydrogenation of acetone and acetophenone. Adkins' catalyst was more active in the hydrogenolysis of acetophenone to ethylbenzene than Cu_2O-V_2O_4-CaO.

G. Of the alkaline earth oxides calcium oxide was the best promoter for Cu_2O-V_2O_4 in the hydrogenation of furfural to furfuryl alcohol and acetophenone to methylphenylcarbinol. Magnesium oxide promoted Cu_2O-V_2O_4 in the hydrogenation of furfural but produced more highly hydrogenated products. The MgO and BaO were inactive as promoters for Cu_2O-V_2O_4 in the hydrogenation of acetophenone. Strontium oxide had some promoter action in both hydrogenations. Barium oxide promoted Cu_2O-V_2O_4 rather remarkably in the hydrogenation of furfural but did not produce complete hydrogenation.

CONCLUSIONS

1. The comparison of catalytic activity of catalysts containing Cu_2O-V_2O_4-CaO required careful drying of each constituent and the control of as many variables as possible.

2. A cuprous oxide catalyst prepared by Menzel's method contained approximately 94 per cent Cu_2O, 4 per cent H_2O, and 2 per cent copper as metal.

3. Calibration of the Parr hydrogenation apparatus was necessary in order to determine the moles of hydrogen actually consumed by the hydrogen acceptor.

4. Increasing the proportion of V_2O_4 in the catalyst Cu_2O-V_2O_4-CaO up to a weight ration of Cu_2O to V_2O_4 of 10 to 5 (optimum) lowered the temperature at which reaction started and increased the rate of hydrogenation of furfural. The addition of V_2O_4 to Cu_2O-CaO decreased the rate of hydrogenation of acetophenone but stabilized the catalyst against reduction.

5. Increasing the proportion of CaO in the catalyst Cu_2O-V_2O_4-CaO was found to be very advantageous in producing an active catalyst. The lowering of the temperature at the start of the reaction and the increase in the rate of the reaction were appreciable.

6. The Cu_2O-V_2O_4-CaO was more active for hydrogenating the aldehyde group than the ketone group in the compounds investigated. This catalyst was not as active as Adkins' copper-chromium oxide catalyst for hydrogenolysis of organic compounds.

7. Calcium oxide was the best alkaline earth oxide used as a promoter for Cu_2O-V_2O_4 in the hydrogenation of furfural and acetophenone.

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THE SYNTHESIS OF *o*-HYDROXYALDEHYDES[1]

LAWRENCE M. LIGGETT

From the Department of Chemistry, Iowa State College

Studies on the synthesis of *o*-hydroxyaldehydes were undertaken in an effort to find a more satisfactory method for the laboratory and commercial preparation of this class of compounds. The *o*-hydroxaldehydes are of interest, being yellow in color, and volatile with steam, properties probably derived from their "hydrogen bridge" structure, and in forming "chelate ring" compounds with the metals. The aldehyde group in this class of compounds is remarkably stable toward alkali and quite resistant to oxidation. It does, however, condense readily with primary amines to give Schiff's bases which in turn form chelate compounds with several metals. Salicylaldoxime, the oxime derivative of the most common of the *o*-hydroxyaldehydes, salicylaldehyde, has found rather extensive use as an analytical reagent.

Nearly all of the *o*-hydroxyaldehydes reported in the literature have been prepared by means of the Reimer-Tiemann reaction. This reaction consists in the action of chloroform on a phenol in a strongly alkaline solution. The method involves several time-consuming operations and gives very low yields. It is particularly unsatisfactory for the preparation of *o*-hydroxyaldehydes from *ortho*-substituted phenols, the yields being in general less than 10 per cent. The method is useless when *o*-halogen- or *o*-nitrophenols are employed. Although many modifications of the Reimer-Tiemann reaction have been suggested previous to this work, no systematic study has been reported of the effect of varying the amounts of reagents used over a considerable range in order to determine the optimum conditions for the reaction. Such a study of the Reimer-Tiemann reaction has now been made using *o*-ethoxyphenol. Two series of reactions were run in which the chloroform concentration was varied from 0.8 to 8.0 moles per mole of phenol. Another series of reactions was conducted in which the only variable was the alkali concentration. As a result of this study the optimum proportions of reagents were found to be 4 moles of chloroform, 9 moles of alkali, and 80–85 moles of water to 1 mole of the phenol. The best yield obtained, however, was only 10 per cent.

A series of reactions was carried out in an autoclave employing pressures of 150–250 p.s.i.g. It was found that the yields by the Reimer-Tiemann method may be increased only a few per cent by carrying out the reaction at a pressure of 150–175 p.s.i.g.

Another method of synthesizing *o*-hydroxyaldehydes, the allyl rearrangement method, has been used to a very limited extent as a laboratory method. This method involves four principal steps, namely, prepara-

[1] Doctoral thesis number 733, submitted November 15, 1943.

tion of the allyl ether of the phenol, rearrangement of the allyl ether to the *o*-allylphenol, isomerization to the *o*-propenylphenol, and finally, oxidation of the propenyl group to an aldehyde. Although this method is somewhat involved the yields obtained over the first three steps in the synthesis are excellent. The oxidation step, however, has been found difficult. The method was considered too long and involved to be a convenient laboratory method.

A new and apparently general method for the preparation of *o*-hydroxyaldehydes was described recently by Duff. This reaction consists in bringing together hexamethylenetetramine and a phenol in the presence of anhydrous glycerol and glyceroboric acid at a temperature of 160°. Initial attempts to use this method in the preparation of several other *o*-hydroxyaldehydes were unsuccessful. Control of temperature in the reaction was not extremely important. The critical step was the manner of adding the phenol and hexamethylenetetramine to the hot glyceroboric acid mixture. Best results were obtained if the two reactants were added simultaneously. Otherwise the phenol, when added alone, polymerized rapidly with the glycerol, and the hexamethylenetetramine if added alone decomposed rapidly at the high temperature. This slight modification in the original procedure resulted in a marked increase in the yields obtained. As a laboratory synthesis the method of Duff was found to be far superior to the Reimer-Tiemann reaction, the time required being only a fraction of that necessary in the Reimer-Tiemann method. The method was found applicable to *ortho*-substituted phenols including halogenated phenols. The yields obtained by the Duff reaction ranged from 10 to 40 per cent, a marked improvement over the Reimer-Tiemann method.

Adopting the modified Duff procedure, the following *o*-hydroxyaldehydes have been prepared: 2-hydroxy-3,6-dimethylbenzaldehyde, 2-hydroxy-4,6-dimethylbenzaldehyde, 2-hydroxy-3,5-dimethylbenzaldehyde, 2-hydroxy-5-methylbenzaldehyde, 2-hydroxy-3-bromo-5-*tert*-butylbenzaldehyde, 2-hydroxy-3-*iso*-propyl-6-methylbenzaldehyde, 2-hydroxy-5-phenylbenzaldehyde, 2-hydroxy-3-bromobenzaldehyde, 2-hydroxy-3-chlorobenzaldehyde, 2-hydroxy-5-ethylbenzaldehyde, 2-hydroxy-3-*n*-butoxybenzaldehyde, 2-hydroxy-3-*tert*-amylbenzaldehyde, 2-hydroxy-3-methyl-5-*tert*-amylbenzaldehyde, 2-hydroxy-3-chloro-5-*tert*-butylbenzaldehyde, 2-hydroxy-3-*iso*-propyl-5-chloro-6-methylbenzaldehyde, and 2-hydroxy-3,5-dibromobenzaldehyde. The procedure was also applied to several phenols from which two different o-hydroxyaldehydes might be formed. It was not definitely established whether the products obtained in these instances were mixtures of the two possible isomers or single compounds. These phenols were as follows: 3,4-dimethylphenol, 2-methyl-4-chlorophenol, 3-methyl-4-*tert*-butylphenol, and 2-hydroxy-4-*tert*-butylphenol.

With the exception of a few aldehydes originally reported by Duff the above aldehydes have not been previously prepared by this method. Several have not been heretofore reported in the literature. The compounds were carefully purified, appropriate physical constants determined, and derivatives prepared.

The Schiff's bases formed by the condensation of these o-hydroxyaldehydes and ethylenediamine are yellow crystalline compounds with sharp melting points and may therefore serve as derivatives for the identification of these aldehydes. These derivatives are more easily prepared and recrystallized than are the other common derivatives such as semicarbazones, oximes, and phenylhydrazones.

Aldehydes were not obtained by the application of the Duff reaction to 2-nitrophenol, 2,4-dinitrophenol, and 2-hydroxypyridine.

OPERATING CONDITIONS FOR OPTIMUM BEHAVIOR OF A CONTINUOUS COUNTERCURRENT, COUNTERGRAVITY EXTRACTION PLANT[1]

WILLIAM LUCIUS MCCRACKEN

From the Department of Chemical Engineering, Iowa State College

Soybeans have increased at a rapid rate in both agricultural and industrial importance during the past few years. While many products are made from soybeans the principal products are the oil and the meal. The oil is used largely for edible purposes and to a lesser extent in paints, printing ink, linoleum, and soap. The meal is a high protein stock feed.

Soybean oil may be pressed out or dissolved out with a suitable solvent. The solvent process removes 95 per cent or more of the approximately 20 per cent oil present in the beans, while the pressure process seldom removes over 75 per cent. Several solvent extraction systems, differing in details, are in commercial use. These systems use commercial hexane as a solvent. Hexane is a good solvent for soybean oil and is low in first cost but, being flammable, presents a definite explosion hazard.

Various nonexplosive solvents have been considered for the extraction of vegetable oils such as soybean. Of these trichloroethylene appears to be the most attractive. It is an excellent solvent for soybean oil. It is nonflammable, low in corrosive action on steel, and easily removed from the oil and meal.

A review of the literature indicates that trichloroethylene vapors have a toxic action, although some of the symptoms ascribed to it may be due to the presence of impurities in the commercial product. By the use of suitable precautions the health hazards may be reduced to a negligible amount.

Analytical methods for the determination of oil in the beans, oil in the meal, moisture in the beans, moisture in the meal, oil in the miscella, trichloroethylene in the oil, and trichloroethylene in the meal have been selected and adapted to the control of the process.

Experimental studies have been carried out in semicommercial equipment described in a thesis by Kircher (1). The extractor consisted of two lengths of steel pipe joined at an angle of about 60 degrees with the longer pipe at an angle of about 15 degrees with the horizontal. The flaked beans moved down the longer pipe countercurrent to the movement of the solvent which entered part way up the shorter leg. The extracted meal was freed of solvent in a jacketed three-section drier. Screw conveyors were used in both the extractor and the drier to move the flakes continuously forward. Two tubular heat exchangers (the evaporator and stripper) were used to remove the solvent from the oil. The solvent vapors from the driers and the stripper were condensed in a surface condenser.

[1] Doctoral thesis number 727, submitted August 3, 1943.

After preliminary runs to check the general operation of the equipment, a series of runs was made to determine desirable changes in equipment and operating procedure. Significant problems and their solutions will be summarized.

Flaking the beans was the first step in the extraction process. Beans cracked into several pieces in a roll crusher were flaked between a pair of smooth rolls. Since some finely powdered material which caused difficulties in operation was always produced during the flaking, studies were made of means of reducing it. Best results were secured from beans tempered by adjusting the moisture and temperature with steam before flaking. This tempering rendered the beans more plastic, thus reducing the fine material to a minimum. A maximum thickness of 15 thousandths of an inch was found desirable for maximum extraction in minimum time.

Even with careful flaking sufficient fine material was carried from the extractor into the evaporator and stripping still to cause difficulty with foaming and bumping and to interfere with the proper stripping of the solvent from the oil. In an effort to remove the fines, a settling tank was installed between the vaporizer and the still. While enough of the fines settled out in this tank to increase the length of time the equipment could be successfully operated, the use of a settling tank, which would require a considerable investment in extra solvent and equipment, was not considered to be a solution of the problem. A new arrangement of the vaporizer and the still was made. The kettles at the bottom of the vaporizer and still were removed. The former was placed directly over the latter with a 1-foot-long section of pipe with a side outlet between. The bottom end of the still dipped into a special bucket kept full of oil to act as a seal. Under this new arrangement the miscella filmed down over the tubes in the vaporizer and still, and the oil and the bulk of the fines passed out the bottom into the sealing bucket.

Two miscella filters were eventually installed. Each of these was a bag filter of cloth suspended in a casing of 12-inch pipe with a removable flanged cover. Only one filter was used at a time allowing plenty of time for emptying the other. These filters operated very satisfactorily.

The original screw conveyors in the driers and steamer were of the ribbon type using a ⅜-inch rod instead of the conventional flat ribbon. The wet meal, entering the top drier from the extractor by gravity, accumulated in the inside of the ribbon and rotated with it instead of moving forward. To remedy this about 5 feet of the ribbon screw were removed and replaced with solid flight screw. Similarly about 2 feet of solid flight conveyor was installed at the entry ends of the second drier and the steamer. Later the solid flight in the second drier was reduced to 1 foot following the formation of a plug in this drier, believed to have been caused by too great a length of solid flight.

In the original arrangement the solvent vapors from each of the two driers and the steamers were carried by separate vapor lines to the condenser above the driers. Considerable difficulty was experienced by the clogging of vapor lines and fouling of the condenser with meal dust. It

was found, after several changes, that the most satisfactory arrangement was to use one vapor line of large size connecting the outlet end of the upper drier to the condenser. Since the solvent vapors are heavier than air the condenser was placed below the level of the driers. To prevent the loss of solvent vapor from the steamer, the meal was originally discharged through a homemade barrel valve, but this stuck on several occasions when meal dust coated the moving bearing surface. Attempts to replace the valve by a conical hopper with a bottom discharge gate were unsuccessful. The problem was solved by procuring a commercially made barrel valve which functioned excellently.

The flaked beans were originally fed into the extractor at a point below the liquid level, but experience showed that more satisfactory results could be obtained by feeding in just above the liquid. A rotameter for measuring the solvent going into the extractor was found to be desirable.

Operation of the equipment under good conditions indicated that the oil content of the meal could be reduced to 1 per cent and the solvent loss to less than 1 per cent of the beans processed. The steam required was about 1 pound per pound of beans, and the water required was about 1.5 gallons per pound of beans.

It is believed that commercial equipment designed on the basis of the data secured could be operated successfully for processing soybeans.

LITERATURE CITED

1. KIRCHER, C .E.
1940. Development of a plant for countercurrent extraction of soybeans with trichloroethylene. Doctoral Thesis, Iowa State College.

CHANGES IN PALATABILITY, MICROSCOPIC APPEARANCE, AND ELECTRICAL RESISTANCE IN BEEF DURING THE ONSET AND PASSING OF RIGOR AND DURING SUBSEQUENT STORAGE[1]

PAULINE CONSTANCE PAUL

From the Department of Foods and Nutrition, Iowa State College

A study was made of the effect of storage at 34–36°C. on a yearling steer, and of the differences between the principal muscles of the round of this animal. The storage times used were 0, 1, 2, 4, 9, and 18 days. The muscles utilized were the semitendinosus, semimembranosus, biceps femoris, the three vasti, adductor, and gastrocnemius of the round, and the psoas major of the loin. A balanced incomplete block design was employed in assigning the storage times to the various cuts and in analyzing the data.

The muscles were separated, cut into roasts, wrapped in pliofilm and stored in the Animal Husbandry meat cooler. After the appropriate period of storage, they were taken out for testing. The raw roasts were inspected for any changes due to storage and for differences between the muscles. The electrical conductivity and the pH were determined for the raw meat. The cuts were then roasted in 150°C. ovens. The maximum internal temperature of the roasts averaged 66°C., with a range of 65 to 67°C. The appropriate weights and measurements were taken so that the total, dripping, and evaporation losses, change in volume, and cooking time per pound could be calculated.

After the internal temperature started to drop, the roasts were sampled for judging and objective tests. The palatability factors of tenderness, juiciness, aroma, and flavor of lean and fat were scored by four judges. The objective tests included the force required to shear a cylinder of meat 1 inch in diameter, and the amount of fluid expressible by 250 pounds pressure in 5 minutes.

Samples of both raw and cooked meat were made into microscopic slides for histological study. Cross and longitudinal sections were made of gelatin-imbedded tissues, using the freezing microtome, and of paraffin-imbedded tissues, using the rotary microtome. The frozen sections were stained to differentiate between the muscle fibers and the fat, while the paraffin sections were stained to show the muscle fibers and the two types of connective tissue—collagenous and elastic.

The slides were inspected for changes produced by cooking muscle not yet in rigor, by normal rigor, and by aging in cold storage. The frozen cross sections were used for measurement of the fiber diameters and counts for the number of fibers per bundle in the various muscles.

Supplementary studies were made on a pair of psoas major cuts to

[1] Doctoral thesis number 724, submitted July 9, 1943.

check on the effect of massage before the muscle went into rigor, and on small strips of neck muscle to check the effect of exposure to 70°C. for various times in producing heat rigor.

Inspection of the raw roasts showed that the amount of leakage of juice was greatest during the second to fourth days of storage. By the eighteenth day, the roasts were sticky on the surface and rather "high" in aroma, indicating bacterial activity. This was most noticeable in the vasti, which also attained the highest pH.

The grain and amount of external and internal fat varied from muscle to muscle. The psoas major was the finest in texture, the gastrocnemius and vasti were next, the semitendinosus and adductor were medium grained, and the semimembranosus and biceps femoris were coarse. Only the semitendinosus and the biceps femoris had a continuous covering of external fat on one side. The gastrocnemius had some, and the other muscles very little external fat.

The electrical resistance of the raw meat dropped sharply with storage, while the pH first decreased, then increased as the storage time lengthened.

Only one of the fresh roasts, the psoas major, went into rigor before being cooked. It had passed through the stage of maximum stiffness by the end of the cooking period. The other fresh roasts were not in rigor before cooking, but were in rigor when cooked. All the stored roasts had passed out of rigor before being cooked.

The total cooking losses did not vary significantly with increased storage or between the muscles. The amount of dripping loss increased sharply from the ninth to the eighteenth day of storage, but this was offset by a corresponding decrease in the loss by evaporation. The muscles with large amounts of external fat had somewhat higher dripping losses than those with little or no outside fat covering.

The volume of the roasts decreased with cooking. The cooking time was not changed by storage, but the smaller roasts required more minutes per pound than the larger ones.

The judging scores for tenderness, juiciness, flavor of lean, and aroma increased generally from 0 to 9 days of storage, then either remained about stationary or decreased somewhat. The decrease in scores for flavor of lean and aroma after 18 days of storage was due to the development of "gaminess" in the meat which was considered definitely undesirable by some of the judges. When judgments of the flavor of the fat were possible, the scores indicated little change up to 9 days of storage, with a decided decrease in desirability after 18 days of storage. This decrease was due to the development of rancidity.

The force required to shear the meat decreased with storage, the drop being especially marked from the fresh roasts to those stored 1 day. The press fluid dropped during the early part of the storage period, then increased decidedly during the last 9 days.

The variation in tenderness between the different muscles as indicated by judging scores and by shear showed that the psoas major was

the most tender muscle used, the gastrocnemius and adductor being next. The four large muscles of the round, the semitendinosus, semimembranosus, vasti, and biceps femoris, did not differ significantly from each other in tenderness.

The diameter of the muscle fibers decreased with cooking but not with storage. The variation between the muscles was quite large, the biceps femoris, vasti, and gastrocnemius having the largest fibers and the semitendinosus and psoas major the smallest. The number of fibers per bundle was too variable to show any consistent change with storage or between the different muscles.

Microscopically, the freshly-killed meat had straight to slightly wavy fibers, which were poorly differentiated. Storage for 1 day led to the appearance of rigor nodes and crinkled or kinked fibers. The nodes persisted throughout the storage time, but the crinkles and kinks tended to disappear, being replaced by sharp breaks in the fibers. The stretched areas of the fibers immediately adjacent to the rigor nodes frequently disintegrated, leaving a granular residue. Cooking brought on the appearance of rigor in the fresh meat, and increased the number of broken fibers in the meat stored 9 and 18 days. The rigor nodes produced in the fresh meat by cooking were not as dense as those found in the stored meat.

The change in collagen caused by cooking could be observed in the slides as a loss of ability to take the acid fuchsin stain and a change from the normal fibrous state to a somewhat granular residue.

Massaging the freshly-killed muscle before cooking apparently speeded up the onset of rigor, but caused little other change except in tenderness, the score of the massaged roast being 3.25 points lower, and the shearing force 18 pounds higher, than that of the control.

Exposure of small strips of fresh muscle to 70°C. gave the typical picture of extreme heat rigor, the fibers showing alternate bands of maximum contraction and rarefaction. Two seconds exposure to this heat affected only the outer fibers, but within 10 seconds the fibers were changed throughout the sample of tissue.

DERIVATIVES OF PHENOTHIAZINE AS CHEMOTHERAPEUTIC AGENTS[1]

DAVID ALLEN SHIRLEY

From the Department of Chemistry, Iowa State College

Derivatives of phenothiazine have been selected for study of their chemotherapeutic properties because of the low cost of phenothiazine, its low toxicity to higher animals, and its high toxicity to lower animals, and the fact that in several cases phenothiazine has already been shown to possess chemotherapeutic value. Chief interest in this investigation has been centered on the antimalarial properties of phenothiazine derivatives.

The structural formula of phenothiazine with the numbering system in present use is given in Fig. 1. A survey of the known substitution re-

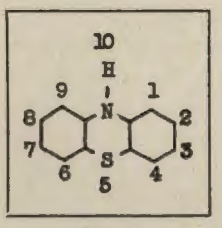


Fig. 1

actions of phenothiazine and 10-substituted derivatives shows that substitution occurs generally in the 3-position. The only exception to this rule previously known is the metalation of 10-ethylphenothiazine with *n*-butyllithium.

A survey of the biological applications of phenothiazine indicates that it has found satisfactory use as an insecticide, as a urinary antiseptic, and as an anthelmintic agent in human and veterinary medicine. A few derivatives of phenothiazine have shown activity as antimalarial agents.

In this investigtion, phenothiazine was shown to undergo metalation by *n*-butyllithium in the 1-position in a yield of 52 per cent. The reactions involved in the proof of the position of the entering lithium atom are shown in Fig. 2. The identity of the ketones VIII and XIII indicates that the position of the entering metal atom was *ortho* to the nitrogen atom.

The metalation of 10-phenylphenothiazine and 10-ethylphenothiazine by *n*-butyllithium was shown to involve either the 2- or the 4-position in the phenothiazine nucleus. The metalation reaction and the reactions used in proving the positions of the entering lithium atoms are shown in Figs. 3 and 4. In the case of the metalation of 10-phenylphenothiazine, the possibility of metalation in the *meta* position on the 10-phenyl group was excluded by the synthesis of this acid and a comparison of its properties with those of the metalation acid. Of the two possible positions for the

[1] Doctoral thesis number 735, submitted December 14, 1943.

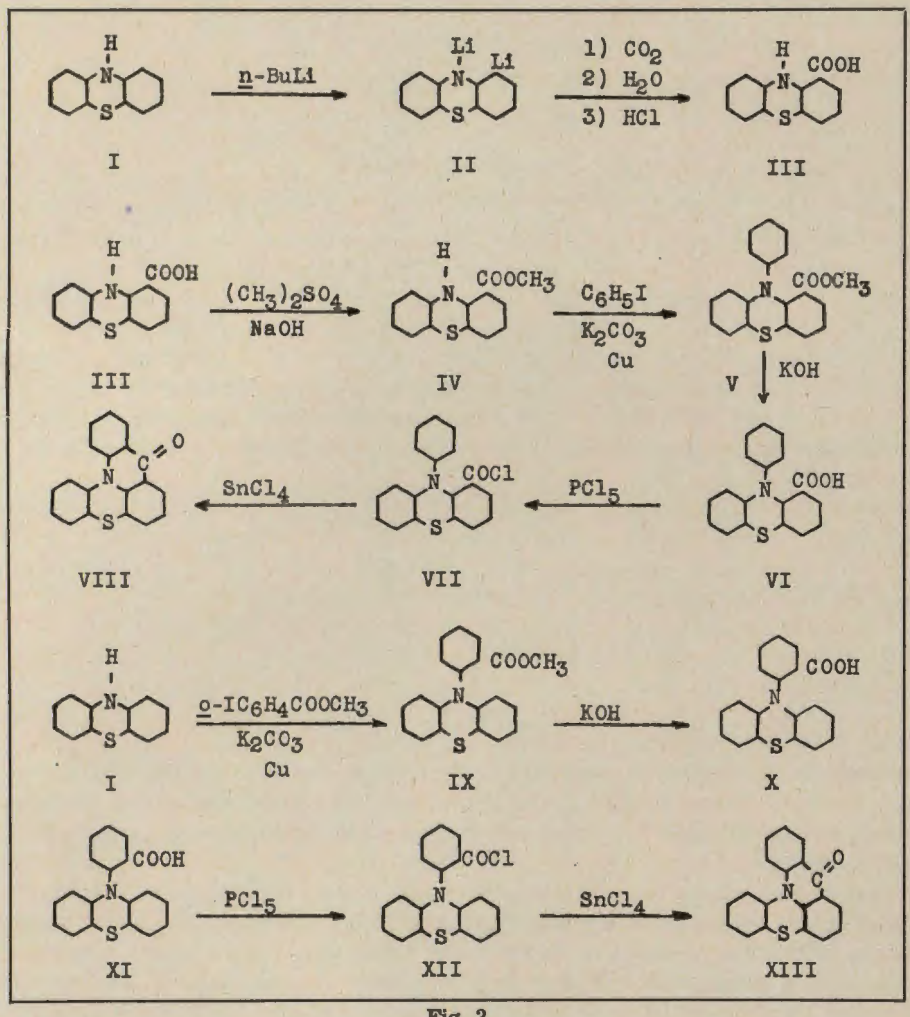

n-BuLi
1) CO2
2) H2O
3) HCl
(CH3)2SO4
NaOH
C6H5I
K2CO3
Cu
KOH
SnCl4
PCl5
o-IC6H4COOCH3
K2CO3
Cu
KOH
PCl5
SnCl4
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII

Fig. 2

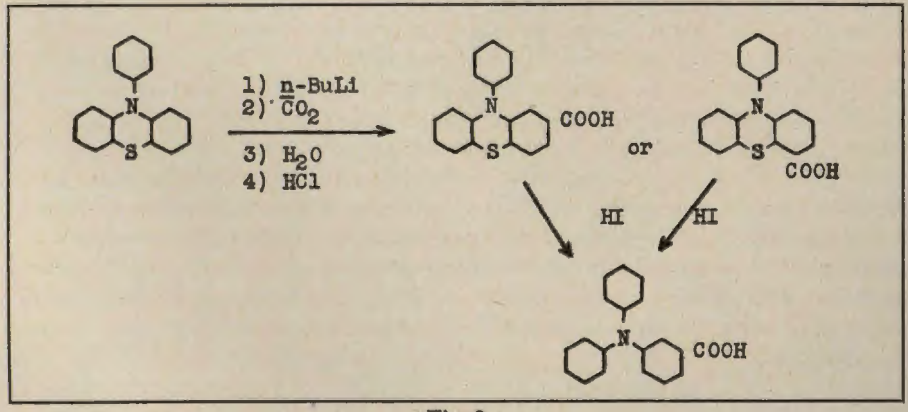

1) n-BuLi
2) CO2
3) H2O
4) HCl
COOH
or
COOH
HI
HI
COOH

Fig. 3

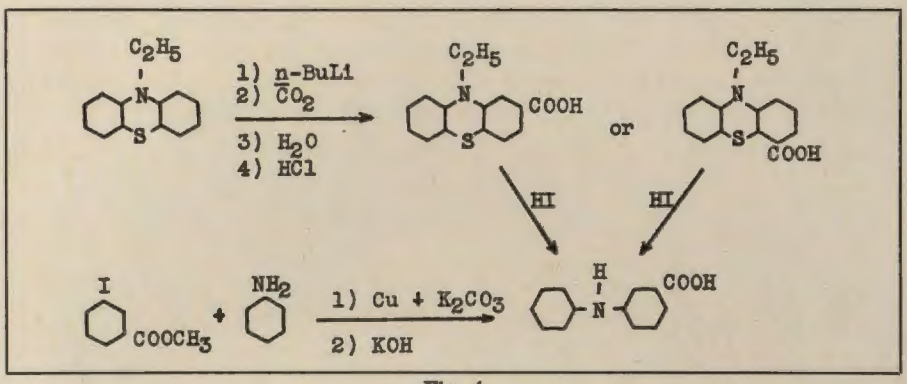


Fig. 4

metalation of 10-phenylphenothiazine and 10-ethylphenothiazine, the 4-position, or the one *ortho* to the sulfur atom, is considered the more likely because of the tendency in metalation reactions with *n*-butyllithium of the lithium atom to enter a heterocyclic nucleus in a position *ortho* to the hetero atom.

The derivatives of phenothiazine which were prepared for pharmacological testing may be divided into the following three groups: (1) derivatives of 10-phenylphenothiazine which contains a γ-diethylaminopropylamino group on the 10-phenyl radical, (2) derivatives of phenothiazine containing a dialkylaminoalkyl group in the 10-position, and (3) miscellaneous derivatives, some of which were prepared as intermediates in the preceding syntheses.

The derivatives of 10-phenylphenothiazine containing the diethylaminopropylamino group were synthesized by the series of reactions shown in Fig. 5, in which the preparation of 10-(2′-γ-diethylaminopropylamino-4′-methoxy)phenylphenothiazine serves as an example. The compounds synthesized by these reactions were 10-(2′-γ-diethylaminopropylamino) phenylphenothiazine (b.p. 215–230°/0.5 mm.), 10-(2′-γ-diethylaminopropylamino-4′-methyl)phenylphenothiazine (the product distilled at a bath temperature of 270° at a pressure of less than 0.5 mm.), 10-(4′-γ-diethylaminopropylamino)phenylphenothiazine (the product distilled at a bath temperature of 350° at a pressure of less than 0.5 mm.), 10-(2′-γ-diethylaminopropylamino-4′-methoxy)phenylphenothiazine (b.p. 220–235°/0.5 mm.), and 10-(2′-γ-diethylaminopropylamino-4′-chloro)phenylphenothiazine (b.p. 270–280°/2 mm.). All of these compounds were yellow, fluorescent, viscous oils. None showed definite antimalarial activity.

The series of 10-dialkylaminoalkylphenothiazine derivatives was prepared by the reactions shown in Fig. 6 in which the preparation of 10-γ-diethylaminopropylphenothiazine serves as an illustration. The compounds prepared by these reactions were 10-β-diethylaminoethylphenothiazine (b.p. 161–165°/0.5 mm.), 10-β-di-*n*-propylaminoethylphenothiazine (b.p. 225–230°/1 mm.), 10-β-morpholinoethylphenothiazine (m.p. 74.5–75.5), 10-β-(6′-methoxy-8′-quinolyl)aminoethylphenothiazine (m.p. 118.5–

119.5°), 10-γ-diethylaminopropylphenothiazine (b.p. 170–182°/0.5 mm.), 10-γ-di-*n*-propylaminopropylphenothiazine (b.p. 257–262°/2 mm.), 10-γ-diallylaminopropylphenothiazine (b.p. 245–260°/1 mm.), 10-γ-piperidylpropylphenothiazine (b.p. 255–265°/1–2 mm.), 3-methoxy-10-γ-di-*n*-pro-

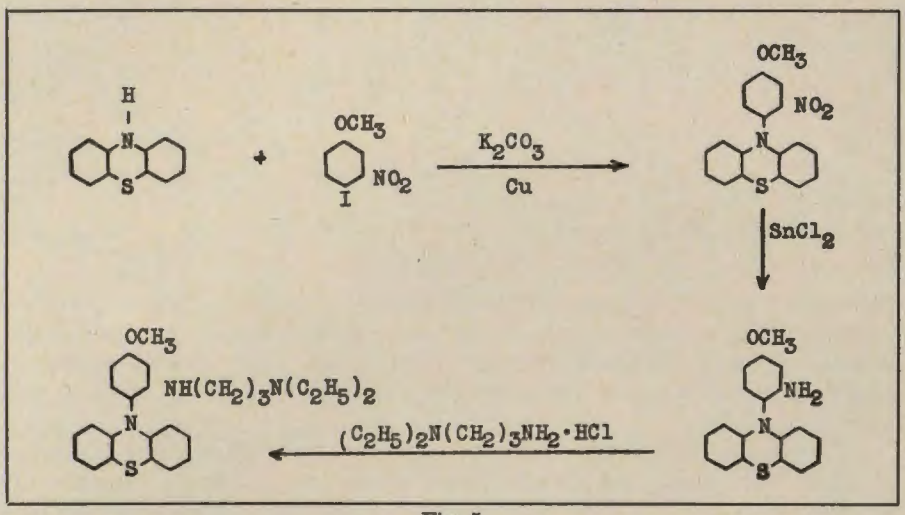


Fig. 5

pylaminopropylphenothiazine (b.p. 250–265°/2 mm.), and 3-methoxy,10-γ-diethylaminopropylphenothiazine (b.p. 220–225°/0.5 mm.). This series of compounds was also without antimalarial activity.

The miscellaneous derivatives of phenothiazine which were not

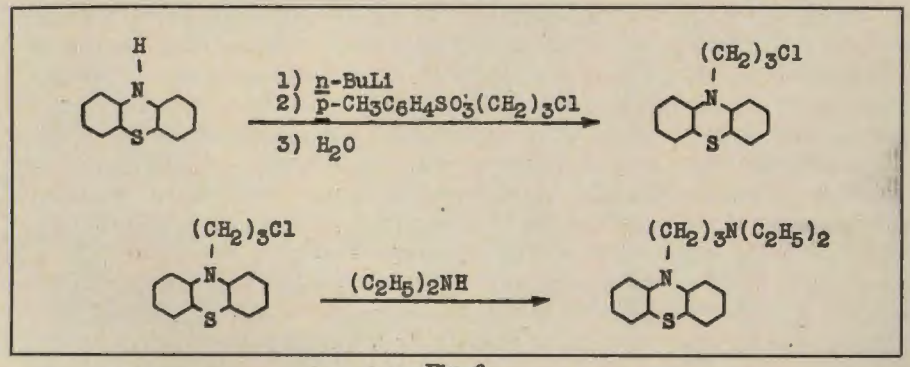


Fig. 6

included as intermediates in the above syntheses are 3-methylphenothiazine (m.p. 160–162°), 1-carboxyphenothiazine (m.p. 264–264.5°), 10-(4′-carboxy)phenylphenothiazine (m.p. 221–221.5°), 3-nitro-10-phenylphenothiazine-5-oxide (m.p. 223.5–224.5°), 10-β-chloroethylphenothiazine (m.p. 96–97°), 10-allylphenothiazine (b.p. 187–195°/1 mm.), 10-decyl-

phenothiazine (b.p. 183–185°/0.5 mm.), and 10-octadecylphenothiazine (m.p. 53°).

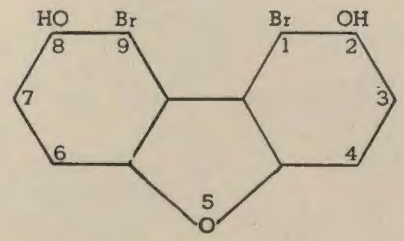
In spite of a low toxicity to higher animals and a high toxicity to lower animals found in the parent compound, derivatives of phenothiazine prepared in this investigation showed no definite activity when tested against malarial parasites in the avian blood stream.

1,9-DERIVATIVES OF DIBENZOFURAN[1]

JOHN ROBSON THIRTLE

From the Department of Chemistry, Iowa State College

Bromination of 2,8-dihydroxydibenzofuran by Swislowsky[2] gave the supposed 1,9(?)-dibromo- derivative (I). Many of the following transformations were made in attempts to prove or disprove this structure.



I

2,8-Dihydroxydibenzofuran was dibrominated by the method of Swislowsky[2] and, wtihout purification, the product was acetylated giving 1,9(?)-dibromo-2,8-diacetoxydibenzofuran. 1,9(?)-Dibromo-2,8-dimethoxydibenzofuran was nitrated in acetic acid at 85°, giving *1,9(?)-dibromo-2,8-dimethoxy-3(?)-nitrodibenzofuran,* m.p. 243–244°. The same product was obtained by the use of acetyl nitrate in acetic anhydride. 1,9(?)-Dibromo-2,8-dimethoxydibenzofuran in acetic acid was refluxed with two equivalents of nitric acid for 1½ hours. A small yield of *1,9(?)-dibromo-2,8-dimethoxy-3,7(?)-dinitrodibenzofuran,* m.p. 222–223°, was obtained. Heating 1,9(?)-dibromo-2,8-dimethoxydibenzofuran on a steam bath with a mixture of equal volumes of acetic acid and fuming nitric acid gave *1,9(?)-dibromo-2,8-dimethoxy-3,4,7(?)-trinitrodibenzofuran,* m.p. 212–214°.

2,8-Dimethoxydibenzofuran was heated with fuming nitric acid, giving 2,8-dimethoxy-1,3,7,9(?)-tetranitrodibenzofuran, m.p. 246–247°, which had been made previously by a different method[3]. The latter was reduced by Raney nickel and hydrogen at 4 atmospheres, giving *2,8-dimethoxy-1,3,7,9(?)-tetra-aminodibenzofuran,* m.p. 181–182°. Acetylation of the tetra-amino-compound gave *1,9(?)-diamino-2,8-dimethoxy-3,7(?)-diacetaminodibenzofuran,* m.p. 295–296°.

1,9(?)-Dibromo-2,8-dimethoxy-3(?)-nitrodibenzofuran was demethylated by hydrobromic acid in glacial acetic acid, giving *1,9(?)-dibromo-2,8-dihydroxy-3(?)-nitrodibenzofuran,* m.p. 267–268°. The latter was nitrated in glacial acetic acid giving *1,9(?)-dibromo-2,8-dihydroxy-3,7(?)-*

[1] Doctoral thesis number 736, submitted December 14, 1943.
[2] Swislowsky, Doctoral Dissertation, Iowa State College. (1939).
[3] Willis and Yeoman, unpublished studies.

dinitrodibenzofuran, m.p. 204°. The same product was obtained by direct dinitration of 1,9(?)-dibromo-2,8-dihydroxydibenzofuran.

2,8-Dimethoxydibenzofuran was mononitrated in acetic acid giving *1(?)-nitro-2,8-dimethoxydibenzofuran,* m.p. 158–159°, and *3(?)-nitro-2,8-dimethoxydibenzofuran,* m.p. 172–174° in the ratio of approximately four parts of the latter to one of the former.

2,8-Dihydroxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid[4] was treated with diazomethane, giving *2,8-dimethoxy-3,7(?)-dibromo-1,9(?)-dicarbomethoxydibenzofuran,* m.p. 230–231°, which was saponified by sodium methoxide and water in methanol to give *2,8-dimethoxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid,* m.p. 322–324°. Decarboxylation of the latter by heat was not successful. 2,8-Dihydroxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid in dioxan was esterified by methanol and anhydrous hydrogen chloride giving *2,8-dihydroxy-3,7(?)-dibromo-1,9(?)-dicarbomethoxydibenzofuran,* m.p. 268–269°. Decarboxylation of 2,8-dihydroxy-3,7(?)-dibromodibenzofuran-1,9(–)-dicarboxylic acid using copper and quinoline resulted in removal of the bromine atoms also, giving 2,8-dihydroxydibenzofuran.

2,8-Dimethoxydibenzofuran-1,9(?)-dicarboxylic acid[2] was treated with fuming nitric acid. The product was a mixture, melting at 247–249°, after two crystallizations from dilute acetone. Esterification of this crude product by methanol and dry hydrogen chloride gave *2,8-dimethoxy-3,4,6,7(?)-tetranitro-1,9(?)-dicarbomethoxydibenzofuran,* m.p. 199.5–200°.

A caustic fusion of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran followed by acetylation gave the acetoxy- compound, melting at 174.5–175.5°, which was converted to a methoxy- derivative, melting at 100–101°. The products were not identified.

1,9(?)-Dibromo-2,8-dimethoxydibenzofuran was treated with one equivalent of butyllithium and then with water giving *3-bromo-2,8-dimethoxydibenzofuran,* m.p. 117.5–118°, whose structure has recently been proved.[5]. When the reaction was repeated using dimethyl sulfate in place of water, *1-methyl-2,8-dimethoxy-7(?)-bromodibenzofuran,* m.p. 144–145°, was obtained. The same product (mixed m.p.) was obtained previously.[5]. These reactions indicated that the supposed 1,9-derivative is, probably, 1,7-dibromo-2,8-dimethoxydibenzofuran.

2,8-Dihydroxydibenzofuran was converted to *2,8-diallyloxydibenzofuran,* m.p. 70–71°, and to *2,8-dibenzofuryloxyacetic acid,* m.p. 271–273°. One attempt to rearrange the allyloxy- compound was not successful.

2-Amino-3-bromodibenzofuran was brominated giving *1(?),3-dibromo-2-aminodibenzofuran,* m.p. 181.5–182°. The same product was obtained by direct bromination of 2-aminodibenzofuran. Diazotization of the amino group and replacement by the hydroxyl group gave *1(?),3-dibromo-2-hydroxydibenzofuran,* m.p. 112–113°. Bromination of 2,8-

[4] Yeoman, unpublished studies.
[5] Hogg, unpublished studies.

diaminodibenzofuran gave polybromo derivatives which could not be purified.

3-Nitrodibenzofuran gave, when incompletely reduced by hydrogen and Raney nickel, 3,3′-azodibenzofuran, m.p. 268–270°. Borsche and Schacke[6] reported a melting point of 282°.

3-Aminodibenzofuran was refluxed with *o*-chloronitrobenzene, potassium carbonate, copper bronze, and nitrobenzene giving *o-nitrophenyl-3-dibenzofurylamine,* m.p. 139.5–140°. 3-Aminodibenzofuran was treated with chloroacetic acid and sodium hydroxide to give *3-dibenzofurylglycine,* m.p. 139–142°, which was esterified to *3-dibenzofurylglycine methyl ester,* m.p. 123–124°.

1,2,4-Trimethoxybenzene was metalated by butyllithium and carbonated to give 2,3,6-trimethoxybenzoic acid, m.p. 149–150°. This acid was previously synthesized by Smith and LaForge[7] and was found to melt at 145–146°. A mixture of these two products melted at 146–149°. The acid was esterified to *methyl 2,3,6-trimethoxybenzoate,* m.p. 57–57.5°, and to *ethyl 2,3,6-trimethoxybenzoate,* m.p. 42.5–43°.

Iodination of the metalation product of 1,2,4-trimethoxybenzene gave *2,3,6-trimethoxyiodobenzene,* m.p. 108–108.5°, which was coupled by the Ullmann reaction to give *2,2′,3,3′,6,6′-hexamethoxybiphenyl,* m.p. 125–125.5°. The latter compound was nitrated by acetyl nitrate in acetic anhydride to give *2,2′,3,3′,6,6′-hexamethoxy-5,5′-dinitrobiphenyl,* m.p. 151–151.5°.

2,3,6-Trimethoxyiodobenzene was nitrated to give *2,3,6-trimethoxy-5-nitroiodobenzene,* m.p. 119.5–120°, which, on shaking with hydrogen and palladium-calcium carbonate catalyst, gave the known 2,4,5-trimethoxynitrobenzene[8] (mixed m.p.). Coupling 2,3,6-trimethoxy-5-nitroiodobenzene using copper powder gave 2,2′,3,3′,6,6′-hexamethoxy-5,5′-dinitrobiphenyl (mixed m.p.).

Ring-closure attempts on the hexamethoxybiphenyl derivatives were unsuccessful.

[6] Borsche and Schacke, Ber., 56:2498 (1923).
[7] Smith and LaForge, Jour. Am. Chem. Soc., 53:3072 (1931).
[8] Schuler and Thoms, Arch. Pharm., 245, 267, 276 (1907). [*Chem. Zentr.*, *II*, 806 (1907)].

FREE RADICALS IN THE DECOMPOSITION OF ORGANOMETALLIC COMPOUNDS[1]

LAUREN A. WOODS

From the Department of Chemistry, Iowa State College

Organometallic compounds undergo thermal, electrolytic, and photochemical decompositions. Free radicals exist as intermediate products in these decompositions. Coupling, disproportionation, and formation of the RH compound by the abstraction of hydrogen from the solvent, are the principal reactions of the free radicals. Most coupling reactions, using metal salts, are considered to operate through free radicals.

Metal-metal interconversions are characteristic of many organometallic derivatives. The reaction is represented by the following general equation:

$$RM^1 + R'M^2 \rightarrow R'M^1 + RM^2$$

Both silver bromide and silver iodide reacted with dimethylmagnesium to give good yields of pure ethane. The reaction was very rapid.

Methyllithium with gold tribromide produced a 76.8 per cent yield of ethane and an 18.4 per cent yield of methane. Since other experiments have shown that trimethylgold normally decomposes to give pure ethane, the methane undoubtedly resulted because of a catalytic action which altered the mode of breakdown of the trimethylgold.

Zirconium tetrachloride reacted with both methylmagnesium iodide and dimethylmagnesium to give pure methane.

With methylmagnesium chloride, tantalum pentachloride formed a good yield of pure methane.

Dimethylmagnesium reacted with chromium trichloride to give pure methane (62.0 per cent yield).

A catalytic amount of gold tribromide (5 mole per cent) effected a coupling of methyllithium with methyl iodide to give ethane. Some methane was produced in the process, but the formation of that compound was probably due to a side-reaction.

Ferric chloride, ferrous chloride, and nickelous chloride catalyzed the reaction of bromobenzene with phenylmagnesium bromide to give yields of biphenyl varying between 26 and 45 per cent based on the total amount of phenyl groups available.

Refluxing dimethylgold bromide in ether for 48 hours produced pure ethane, metallic gold, methylgold dibromide, and gold tribromide. A disproportionation of dimethylgold bromide undoubtedly occurred, giving trimethylgold, methylgold dibromide, and gold tribromide. Being thermally unstable, trimethylgold then decomposed to give pure ethane and metallic gold.

Diphenylcadmium, refluxed in benzene for 82 hours, thermally de-

[1] Doctoral thesis number 723, submitted July 8, 1943.

composed to give only a small yield (3.3 per cent) of biphenyl. However, cadmium chloride reacted with benzylmagnesium chloride to give a 77 per cent yield of the coupling product, bibenzyl.

The preparation of diphenylantimony chloride by reacting antimony trichloride with phenylmercury bromide was attempted. The experiment was unsuccessful and a quantitative recovery of the phenylmercury bromide was realized.

The following satisfactory preparation of diphenylantimony chloride was developed. Triphenylantimony was dearylated by reaction with hydrogen peroxide[2] to give diphenylstibonic acid. This stibonic acid was dissolved in hot, moderately concentrated hydrochloric acid to give diphenylantimony trichloride, the insoluble impurities were removed by filtration, and the diphenylantimony trichloride reduced to diphenylantimony chloride with stannous chloride. The diphenylantimony chloride was purified by crystallization from acetic acid. This process is more dependable and more simple than other preparations of diphenylantimony chloride.

The preparation of phenyl-*p*-tolylstibonic acid by the reaction of phenylantimony oxide with benzenediazonium chloride, and the subsequent alkaline decomposition, was attempted. The desired product could not be isolated.

Tri-*p*-chlorophenylantimony was prepared in a 71.5 per cent yield by the reaction of *p*-chlorophenylmagnesium bromide with antimony trichloride. The compound was crystallized from a chloroform-methanol solution, and melted at 101.0–101.5°.

Tri-*p*-chlorophenylantimony dichloride was prepared in almost a quantitative yield by slowly bubbling chlorine gas through a solution of tri-*p*-chlorophenylantimony in ice-cold chloroform. The product was crystallized from a chloroform-methanol solution. The compound melted at 189.5–190.5°.

Tri-(*p*-dimethylaminophenyl) antimony was prepared in a 35 per cent yield by the reaction of *p*-dimethylaminophenyllithium with antimony trichloride. The crystallization was carried out in an ethanol-chloroform solution. The maximum melting point of the compound was 239–241°.

Both triphenylantimony dichloride and tri-*p*-tolylantimony dichloride were quantitatively reduced by hydrazine hydrate[3] in 95 per cent ethanol to give triphenylantimony and tri-*p*-tolylantimony, respectively.

Hydrazine hydrate apparently did not react with diphenylantimony chloride and *p*-tolylantimony dichloride to give the R_3Sb compounds.

Metal-metal interconversions were carried out on symmetrical and unsymmetrical triarylantimony compounds in accordance wih the following equation:

$$R_3Sb + n\text{-}C_4H_9Li \rightarrow R_2SbC_4H_9\text{-}n + RLi$$

[2] Goddard, "Organometallic compounds," which constitutes Part III of Vol. XI of Friend, "A text-book of inorganic chemistry," Charles Griffin Co., London (1936), p. 239.

[3] Hydrazine hydrate had previously been used to convert arylbismuth halides to R_3Bi compounds by Gilman and Yablunky. Jour. Am. Chem. Soc., 62:665 (1940).

The amount of RLi compound was determined by carbonation to give the corresponding carboxylic acid. The solution of 0.005 mole of the antimony compound and 0.005 mole of *n*-butyllithium in 60 cc. of anhydrous ether was stirred for 10 minutes before carbonation. These conditions were comparable to those used in the experiments with the corresponding organobismuth compounds[4].

Triphenylantimony gave benzoic acid in yields of 8.3, 11.4, and 9.8 per cent. Tri-*p*-tolylantimony produced 5.9 and 6.5 per cent yields of *p*-toluic acid. Tri-*p*-chlorophenylantimony gave *p*-chlorobenzoic acid in yields of 38.0 and 36.9 per cent. Diphenyl-α-naphthylantimony gave a 15.5 per cent yield of α-naphthoic acid; only a trace of benzoic acid was formed. Diphenylmesitylantimony produced a 10.2 per cent yield of benzoic acid; no 2,4,6-trimethylbenzoic acid was produced. Diphenyl-*p*-chlorophenylantimony gave a 20.2 per cent yield of *p*-chlorobenzoic acid, and a 3.9 per cent yield of benzoic acid.

The arrangement of the series of radicals in the decreasing amount of cleavage is as follows: *p*-chlorophenyl, α-naphthyl, phenyl, *p*-tolyl, and mesityl. The series is in entire accord with that based on the cleavage of the unsymmetrical triarylbismuth compounds.[4b]

On the basis of cleavage from unsymmetrical mercurials by hydrogen chloride, the above radicals possess the following order of decreasing lability: mesityl, α-naphthyl, *p*-toyl, phenyl, and *p*-chlorophenyl[5]. It is very apparent that a cleavage series of this type depends upon both the metal derivative being cleaved and the cleaving agent.

The appearance of a transitory green color in the reaction of the liquid ammonia solution of diphenylantimony iodide with metallic sodium may be taken as evidence for the formation of diphenylantimony.

$$(C_6H_5)_2SbI + Na \rightarrow (C_6H_5)_2Sb + NaI$$

The same general observations were made with the corresponding bismuth compound[6].

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[4](a) Gilman, Yablunky, and Svigoon, *ibid.*, 61:1170 (1939); (b) Gilman and Yale, *Chem. Rev.*, 30:281 (1942).

[5]Kharasch, Legault, and Sprowls, Jour. Org. Chem., 3:409 (1938); Kharasch and Flenner, Jour. Am. Chem. Soc., 54:674 (1932).

[6]Gilman and Yablunky, Jour. Am. Chem. Soc., 63:212 (1941).

SYNTHETIC DIBENZOFURANS STRUCTURALLY RELATED TO KNOWN ANALGESICS[1]

FREDERICK ALBERT YEOMAN

From the Department of Chemistry, Iowa State College

Emphasis in recent work on dibenzofuran derivatives has been placed upon the synthesis of 1- and 1,9- substituted compounds which might serve as intermediates in the preparation of products containing a bridge between the 1- and 9-positions. Swislowsky[2] brominated 2,8-dimethoxydibenzofuran to obtain two dibromo isomers which he designated tentatively as 1,9(?)-dibromo-2,8-dimethoxydibenzofuran and 2,8-dimethoxy-3,7(?)-dibromodibenzofuran. The work of Willis[3] has provided strong evidence in support of the structure of the latter compound. A considerable portion of the present studies has been devoted to a completion of the proof of structure of 2,8-dimethoxy-3,7(?)-dibromodibenzofuran and to a further investigation of the structure of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran.

Nitration of 2,8-dihydroxydibenzofuran gave 1,3,7,9(?)-tetranitro-2,8-dihydroxydibenzofuran, m.p. 246–247°, which upon methylation yielded 1,3,7,9(?)-tetranitro-2,8-dimethoxydibenzofuran, m.p. 245–246°. A mixture of the dihydroxy and dimethoxy compounds melted at 218–219°,

A modification of the procedure of Swislowsky[2] for the preparation of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran from 2,8-dihydroxydibenzofuran was introduced. The crude 1,9(?)-dibromo-2,8-dihydroxydibenzofuran obtained from bromination of 2,8-dihydroxydibenzofuran was acetylated to give pure 1,9(?)-dibromo-2,8-diacetoxydibenzofuran, m.p. 173–174°, which was methylated directly to yield 1,9(?)-dibromo-2,8-dimethoxydibenzofuran, m.p. 195–196°. The overall yield for the conversion of 2,8-dihydroxydibenzofuran to 1,9(?)-dibromo-2,8-dimethoxydibenzofuran was 44.3 per cent.

When attempts at bromination of 2,8-dimethoxydibenzofuran-1,9(?)-dicarboxylic acid were unsuccessful, the acid was cleaved to 2,8-dihydroxydibenzofuran-1,9(?)-dicarboxylic acid, m.p. 313–314°, which was dibrominated to yield 2,8-dihydroxy-3,7(?)-dibromodibenzofuran-1,9(?)-dicarboxylic acid, m.p. 318–319°. Decarboxylation of the latter acid was attempted by treatment with a suspension of powdered copper in quinoline. No pure product was obtained, although Thirtle[4] obtained from the same reaction a product which may have been crude 2,8-dihydroxydibenzofuran.

Preparation of 1,9(?)-diamino-2,8-dimethoxydibenzofuran was attempted by treatment of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran with cuprous bromide and ammonium hydroxide in a steel bomb. The amine

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[1] Doctoral thesis number 742, submitted March 16, 1944.
[2] Swislowsky, Doctoral Dissertation No. 540, Iowa State College (1939).
[3] Willis, Doctoral Dissertation No. 712, Iowa State College (1943).
[4] Thirtle, Doctoral Dissertation No. 736, Iowa State College (1943).

obtained proved too unstable for purification. Only starting material was recovered from the treatment of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran with sodamide in liquid ammonia.

Conversion of 1,9(?)-dibromo-2,8-dimethoxydibenzofuran to 2,8-dimethoxydibenzofuran-1,9(?)-dialdehyde, m.p. 237–238°, was accomplished in 62 per cent yield by halogen-metal interconversion with *n*-butyllithium followed by treatment with N-methylformanilide. The aldehyde was characterized by preparation of the dioxime, m.p. 243–244°. Oxidation of the aldehyde yielded 2,8-dimethoxydibenzofuran-1,9(?)-dicarboxylic acid, whose dimethyl ester proved identical by mixed melting point with an authentic sample of 1,9(?)-dicarbomethoxy-2,8-dimethoxydibenzofuran. Attempts were made to prepare cyclic compounds from 2,8-dimethoxydibenzofuran-1,9(?)-dialdehyde by condensations with hydrazine and with o-phenylenediamine. Only products of polymeric nature resulted.

The crude product obtained from dibromination of 2-hydroxydibenzofuran[5] was acetylated to yield 1,x-dibromo-2-acetoxydibenzofuran, m.p. 154–155°. Monobromination of 1-bromo-2-hydroxydibenzofuran[5] followed by acetylation yielded a dibromo compound identical with the above 1,x-dibromo-2-acetoxydibenzofuran, thus establishing the location of the bromine atom in the 1-position. Hydrolysis of 1,x-dibromo-2-acetoxydibenzofuran yielded 1,x-dibromo-2-hydroxydibenzofuran, m.p. 181–182°. The 1-bromo-2-hydroxydibenzofuran used in the above monobromination was prepared by a modification of the method of Van Ess[5]. The crude product obtained from the monobromination of 2-hydroxydibenzofuran was acetylated to give 1-bromo-2-acetoxydibenzofuran, m.p. 135–136°, which was then hydrolyzed to pure 1-bromo-2-hydroxydibenzofuran. Anomalous results were obtained in the attempted cleavage of 2-methoxy-3-bromodibenzofuran to yield 2-hydroxy-3-bromodibenzofuran. The alkali-soluble product obtained melted at 168–170° as compared with a melting point of 143–144° reported by Van Ess for an authentic sample of 2-hydroxy-3-bromodibenzofuran prepared from 2-amino-3-bromodibenzofuran. Methylation of the anomalous cleavage product yielded a white, alkali-insoluble compound, m.p. 154–155°, which was obviously not 2-methoxy-3-bromodibenzofuran.

The crude 1(?)-bromo-2,8-dihydroxydibenzofuran obtained from entrainment bromination of 2,8-dihydroxydibenzofuran acetylated to yield 1(?)-bromo-2,8-diacetoxydibenzofuran, m.p. 142–144°. Methylation of the latter compound gave 1(?)-bromo-2,8-dimethoxydibenzofuran, m.p. 102.5–103.5°.

Treatment of 4-dibenzofuryllithium with *n*-butoxymethylpiperidine gave 4-dibenzofuryl-N-piperidinomethane, b.p. 175–180° (0.5 mm.), which gave a picrate of m.p. 177–178°.

From a diazo coupling of *m*-trifluoromethylaniline with 2-hydroxydibenzofuran was obtained 1-(*m*-trifluoromethylphenylazo)-2-hydroxydibenzofuran, m.p. 173–174°, and a similar coupling reaction with 2,8-dihydroxydibenzofuran yielded 1-(*m*-trifluoromethylphenylazo)-2,8-di-

[5] Gilman and P. R. Van Ess, Jour. Am. Chem. Soc., 61:1365 (1939).

hydroxydibenzofuran, m.p. 256–257°. No pure product could be isolated from the reaction of two equivalents of *m*-trifluoromethylbenzenediazonium chloride with 2,8-dihydroxydibenzofuran.

Treatment of *m*-trifluoromethylphenylmagnesium bromide[6] with N-methylformanilide gave *m*-trifluoromethylbenzaldehyde, b.p. 64–66° (10 mm.), n^{20}_D 1.4660, d^{29} 1.300. From the aldehyde was prepared the oxime, b.p. 102–104° (12 mm.), n^{20}_D 1.5128, d^{29} 1.305, and the 2,4-dinitrophenylhydrazone, m.p. 259–260°. Treatment of 4-aminodibenzofuran[2] with *m*-trifluoromethylbenzaldehyde yielded 4-(*m*-trifluoromethylbenzalamino)-dibenzofuran, m.p. 81–83°. From *m*-trifluoromethylbenzaldehyde and *m*-trifluoromethylaniline was prepared 3-(*m*-trifluoromethylbenzalamino)-benzotrifluoride, m.p. 50–51°.

Attempts at purification of the dibenzofuran-4-aldehyde obtained from reaction of 4-dibenzofuryllithium with N-methylformanilide were unsuccessful. From the crude aldehyde was prepared dibenzofuran-4-aldehyde 2,4-dinitrophenylhydrazone, m.p. 301–302°.

Condensation of acetonylacetone with *p*-aminoacetanilide gave N-(*p*-acetaminophenyl)-2,5-dimethylpyrrole, m.p. 207–208°. Hydrolysis of the acetamino compound gave an amine which was too unstable for purification. No pure product was isolated from the attempted diazo coupling of the crude amine with 2-hydroxydibenzofuran.

Treatment of 5-iodotoluhydroquinone dimethyl ether[7] with cuprous cyanide gave 2,5-dimethoxy-*p*-tolunitrile, m.p. 130–131°. Hydrolysis of of the nitrile gave 2,5-dimethoxy-*p*-toluic acid, m.p. 125–126°. The same acid was prepared from 5-iodotoluhydroquinone dimethyl ether by halogen-metal interconversion with *n*-butyllithium followed by carbonation. Oxidation of 2,5-dimethoxy-*p*-toluic acid gave 2,5-dimethoxyterephthalic acid, m.p. 265–265.5°, which was converted to the diethyl ester, m.p. 101–102°. The melting points obtained for 2,5-dimethoxyterephthalic acid and its diethyl ester agree with those reported by Nef[8]. It has thus been demonstrated that iodination of toluhydroquinone dimethyl ether involves the 5-position, and the ring-closure synthesis of 2,8-dihydroxy-3,7-dimethyldibenzofuran by Willis[3] has been validated.

The 5-nitrotoluhydroquinone dimethyl ether reported by Erdtman was reduced to the corresponding amine which proved too unstable for satisfactory purification. Acetylation of the crude amine yielded 5-acetaminotoluhydroquinone dimethyl ether, m.p. 160–162°. The diazonium salt obtained from diazotization of 5-aminotolyhydroquinone dimethyl ether was converted to an iodo compound identical with 5-iodotoluhydroquinone dimethyl ether. Nitration of toluhydroquinone dimethyl ether is therefore shown to involve the 5-position.

Nitration of 5-iodotoluhydroquinone dimethyl ether gave 5-nitrotoluhydroquinone dimethyl ether. Similarly, nitration of 2,5-dimethoxy-*p*-toluic acid resulted in an exchange of groups with the formation of 5-nitrotoluhydroquinone dimethyl ether.

[6] Simons and Ramler, Jour. Am. Chem. Soc., 65:389 (1943).
[7] Erdtman, Proc. Roy. Soc. (London), A143:191 (1933).
[8] Nef, Ann.: 258, 298 (1890).

DEVELOPMENT AND STRUCTURE OF *BROMUS INERMIS* LEYSS[1]

IRVING WILLIAM KNOBLOCH[2]

From the Botany and Plant Pathology Section and the Farm Crops Subsection, Iowa Agricultural Experiment Station

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Bromus inermis Leyss., the smooth brome grass, has attained prominence in recent years as a promising grassland and forage crop. The plant is known by a number of names: Hungarian brome, Austrian brome, Russian brome, awnless brome, and smooth brome. Its native habitat is variously said to be Eurasia, Russia, central Europe, and China. It was introduced into the United States in 1882 and has become a widely distributed and increasingly important crop. A knowledge of its development, morphology, and cytology will have direct application to problems relating to the development of improved strains.

REVIEW OF LITERATURE

Zherebina (75) (76) recognized two principal types of *Bromus inermis*, a steppe type and a meadow type. The meadow type was further subdivided into four subtypes: (1) tall, (2) bushy, (3) a prolific seed producer, (4) a type with short culms. The steppe type was found to be inferior to the meadow type in forage value and succulence, but superior in drought resistance. Physiological and morphological variations in seedlings as well as in members of the same clone were noted by Waldron (64). Differences were found in blade length and width, culm height, and crude protein content. Waldron (65) compared the coefficient of variation of smooth brome grass with that of other grasses and found a significant correlation only between height and weight.

Considerable variation in leafiness, height, habit of growth, rhizome development, heat and drought tolerance, disease resistance, and seed-producing qualities of smooth brome grass was noted by Frolick and Newell (27) who believed that much of the variability was environmental. Knobloch (40) found that six of eleven selected morphological characters in smooth brome grass varied to a greater degree than had been previously reported. The six characters were blade width, length of panicle, length of spikelet, lengths of both glumes and length of palea. The extensive literature dealing with variation in other grasses has been reviewed by Knob-

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[2] The writer expresses his appreciation to Dr. J. E. Sass for encouragement and advice throughout the course of the investigation. Thanks are due to Drs. G. J. Goodman and I. J. Johnson for assistance in the preparation of the manuscript and to Dr. C. P. Wilsie for the use of plants from the Iowa Agricultural Experiment Station plots.

loch (40). Beddows (10) reported that smooth grome grass is xenogamous but that pollination occurs between different spikelets of the same plant. After studying 121 strains, Keyser (39) concluded that many of them bred true. Some progenies, however, broke up into further strains.

Growth studies on brome grass have dealt principally with the root system. Weaver (69) found that the roots penetrated to a depth of about 4.7 feet. A depth of 5.5 feet for 2-year-old plants was recorded by Ten Eyck (61). A root length of 39 cm. after 12 months growth was measured by Gruber (30). Root lengths of as much as 287 cm. were found by Witte (73). Kannenberg (35) noted that after 2 years in sandy soil the roots of one plant covered 2.48 square meters.

Watkins (67) studied the effects of fertilizers, shade, competition and photoperiodism on brome grass. Fertilizers increased leaf production, the height and number of shoots and the dry weight of tops, but decreased the total number of rhizomes and the weight of the underground parts. Shade increased the number of elongated internodes, the height of the plants, and the nitrogen content, but lowered the carbohydrate content. A photoperiod of 18 hours increased the height and weight, the size of the rhizomes, and the percentage and absolute amounts of carbohydrates. Short-day plants had more shoots than long-day plants. When sown with alfalfa, the number of shoots and rhizomes and the dry weight increased.

Cytological work on *Bromus inermis* has consisted mainly of surveys of chromosome numbers in races or strains. Knobloch (40) has reviewed the literature on this phase. The species is known to have numerous forty-two-chromosome and fifty-six-chromosome races. One race having seventy-two chromosomes has been reported (49).

Morphological descriptions of Bromus are fragmentary. Sirrine (57) described the anatomy of the leaf in some detail. A cambiform meristematic zone in the nodes was described by Chrysler (21).

MATERIALS AND METHODS

The plants used in this study were obtained from the forage crops breeding nursery at the Iowa Agricultural Experiment Station, Ames, Iowa. These plants had been established from open-pollinated single plant selections and strains.

Strain 543-34 was used for the study of germination, seedling development, and for anatomical characteristics. This strain originated from a single-plant selection made at the Dominion Forage Crops Laboratory, Department of Agriculture, Saskatoon, Saskatchewan, Canada. After selective inbreeding, the third generation inbred progeny of the single plant was increased and distributed under the variety name Parkland.

For the anatomical study of the vegetative organs, most of the material was embedded in paraffin. Excessively hard or brittle tissues were embedded in celloidin. Microtome sections were stained most commonly in safranin-fast green. The hot water method of softening paraffin-embedded tissues made possible the sectioning of most tissues. Most of the drawings were made with the aid of a microprojector.

GERMINATION OF THE CARYOPSIS: DEVELOPMENT OF THE SEEDLING INTO AN ESTABLISHED PLANT

The order of emergence and gross features of the development of the vegetative organs are presented in the following descriptions. The developmental rates should be regarded as applicable only to the greenhouse conditions used in this study. Further studies should be made under controllable greenhouse conditions as well as under a range of field conditions.

First and second day after planting: The first observable evidence of germination is the swelling of the entire caryopsis. The radicle grows and pushes the coleorhiza against the pericarp. The coleorhiza then emerges near the base of the dorsal side of the caryopsis. The protruding coleorhiza bears white hairs, less than a millimeter in length. The primary root breaks out of the thin side of the coleorhiza rather than through the thicker tip and thus projects, for a time at least, at right angles to the median plane of the plumule.

Fifth day: Under the conditions prevailing in these plantings, the primary root was at least 18 mm. long and densely covered with root hairs except at the tip. The first adventitious root had made its appearance from the region of the scutellar node. The coleoptile was 10 mm. long and had developed chlorophyll. The first foliage leaf, 10 mm. long, had developed chlorophyll at the tip and white epidermal hairs on the inner and outer surfaces.

Fourteenth day: The primary root was as much as 80 mm. in length and had eight lateral roots. The scutellar adventitious roots were 30 mm. long and two or three roots had arisen at the coleoptile node. The first internode or "mesocotyl" appeared; the first foliage leaf was 85 mm. and the coleoptile about 20 mm. in length. The lemma, palea, and caryopsis were still adherent to the seedling.

Twenty-first day: The primary root bore numerous lateral roots, frequently in recognizable pairs. The scutellar root was about 37 mm. and the first internode 10 mm. in length. The first leaf had grown to 105 mm. and the second leaf to 72 mm. in length. The parenchymatous ligule on the first leaf was fully developed, 1 mm. long, and located approximately 20 mm. above the coleoptile node.

Twenty-eighth day: The primary root system comprised the largest part of the root system, having as many as thirteen large lateral roots. The largest adventitious root equaled the primary root in diameter, but was only about half as long. The adventitious roots from the coleoptile node emerged by breaking through the enveloping coleoptile.

Fifty-fourth day: The largest adventitious root in greenhouse plants was 150 mm. long; some seedlings had as many as three tillers and five leaves, and the coleoptile had shredded off.

One hundred eleventh day: As many as ten leaves were present, the lower six usually dead. The coleoptile node had become greatly swollen as a result of the formation and emergence of adventitious roots. A study of seedlings with three tillers showed that the best-developed tiller had a ruptured, two-veined prophyllum approximately 5 mm. long. This tiller

had two leaves, each with a distinct sheath 12 mm. long, and a blade 3 mm. long. Each leaf had a small ligule. Four other small, less-developed leaves were present. The second tiller also had a prophyllum 5 mm. in length. The first or basal leaf was approximately 18 mm. long, and the second leaf was at least 23 mm. long. Four other leaves were present, each having a blade and sheath. The third tiller, which was the smallest and youngest, had a prophyllum. The first leaf had a sheath 13 mm. long and a 2 mm. blade. The sheath of the second leaf was 23 mm. long, and the blade 5 mm. long. Roots had emerged from the bases of all three tillers.

One hundred eighty-ninth day: The primary roots were still present, but the remnants of the caryopsis were no longer attached to the plant. Between this date and the two hundred and twenty-first day, two short rhizomes developed, each less than 10 mm. long. The main culm had three tillers at its base, and each rhizome had three tillers at its apex. The plant was now well established, and showed evidence of spreading. No observations were made after this date.

GROSS MORPHOLOGY AND ANATOMY OF THE PLANT

PANICLE

The panicle in the strains studied varies in length from 80 to 200 mm. and consists of a central rachis and numerous secondary and tertiary branches. The panicle is wide-spreading, but occasionally it is unilateral (fig. 1). At maturity the panicle is somewhat contracted and purplish-brown, later changing to straw color. The secondary branches are arranged in fascicles, each fascicle constituting a half-whorl. The tertiary branches may occur singly or in half-whorls, and may branch again before ending in a spikelet. The rachis is usually glabrous, but the secondary and tertiary branches are covered with short, stiff, white, forward-pointing hairs.

Anatomically, the rachis is a hollow cylinder with two circles of vascular bundles, the outer circle having smaller bundles placed directly within the sclerenchymatous hypodermis (fig. 5). The larger bundles of the inner circle usually alternate with the smaller outer ones. Each bundle is composed of two to three protoxylem cells, two large flanking metaxylem vessels, xylem parenchyma, tracheids, phloem, and a thin bundle sheath.

The epidermis is heavily lignified, especially the outer cell walls. Masses of thin-walled chlorenchyma alternate with the thick-walled hypodermal groups around the periphery of the rachis, with stomates opening into the former. More chlorenchyma is found in the rachis than in the lower part of the culm. Bridges of thick-walled cells connect the lignified hypodermal groups under the chlorenchyma. The rest of the cortex is composed of thin-walled parenchyma.

In the secondary and subsidiary branches of the rachis the epidermal cells are also strongly thickened (fig. 6). There are two to three uninterrupted peripheral layers of hypodermal chlorenchyma and three to six layers of thick-walled sclerenchyma, usually containing four small, evenly-

spaced vascular bundles. The center of the branch is occupied by one to two larger bundles.

SPIKELET

The pedunculate spikelet usually ranges from 10–30 mm. in length and consists of a rachilla bearing two glumes, and two to ten florets. The upper one or two florets may be sterile, consisting of only a lemma, or a lemma and a palea, or a lemma and palea with reduced stamens and pistil.

The central axis of the spikelet is a disarticulating rachilla, each segment of which is about 3 mm. long and has a convex and a concave side, the latter facing toward the palea. Most of the rachilla is covered with stiff, white, upward-pointing hairs, and the distal end of each rachilla segment is oblique. At the base of the lemma, a mass of callus occurs, inseparable from and apparently fused to the rachilla.

GLUMES

The first or lower glume varies from 3 to 7 mm. in length and is smooth, acute, lanceolate, and has a prominent keel. Usually, one bundle is present, but occasionally three bundles occur. The chlorenchyma extends over the basal half of the glume, but distally it is confined to the bundle region. Stomates are present on both surfaces.

The internal anatomy of the first glume is similar to that of the palea and lemma (figs. 9–11). The outer or lower epidermal cells are heavily lignified on their rippled outer walls and on the radial walls, but less lignified on their inner tangential walls. Silicified cells occur scattered in the epidermis. The hypodermis may or may not be lignified. Below the hypodermis are one to three layers of thin-walled chlorenchyma. The inner or upper epidermal cells are rectangular and thin-walled. The glume margins are usually two cells in thickness. The bundle contains a few xylem elements, a large mass of phloem, and a bundle sheath which merges with the outer epidermis.

The second glume is broader and longer than the first glume, being from 4–9 mm. in length, and is smooth, acute, and lanceolate. Stomates are present on both surfaces, and the chlorophyll is distributed as in the first glume. The anatomy is similar to that of the first glume except that there are three vascular bundles (figs. 7–8). In the development of the spikelet, the glumes develop before the florets and enclose the rest of the young spikelet, while at maturity the glumes are much shorter than the rest of the spikelet.

LEMMA

The elliptical lemma ranges in length from 7–14 mm., and is thicker and more chlorophyllous than the palea. The lemma is convex on the outside, concave toward the pistil, its margin is translucent, and the surface is glabrous toward the apex and pubescent basally. The tip of the lemma is bifid and may have a chlorophyllous awn arising from the back. This awn is so near the apex that it appears to originate from between the

lemma tips. The awn may be 2 mm. in length. There are five to seven bundles, the center one and each alternate one being larger and hence more conspicuous than those in between (figs. 12–13). This pattern is similar to that of the blade. Chlorenchyma is abundant basally except in the margin, but distally is confined to the bundles. A thick callus occurs at the base of the lemma.

The outer epidermis is heavily lignified, especially on the rippled outer wall. Silicified cells and thickened hairs are also present. The hypodermis consists of one to two layers of cells, somewhat less lignified than the epidermis. Between the hypodermis and inner epidermis, one to three layers of thin-walled chlorenchyma cells extend throughout the interior of the lemma. The inner epidermis consists of rectangular, thin-walled cells without chloroplasts. Stomates occur on both surfaces. The margins of the lemma are composed of a mass of translucent sclerenchyma. The larger vascular bundles are like those of the palea, but the smaller ones have fewer xylem and phloem cells and a narrower bundle sheath.

PALEA

The palea is a bicarinate organ 5–14 mm. in length. It is concave on its dorsal side, and its "wings," each about 0.5 mm. wide, turn inward sharply, being concave on the ventral side (fig. 14). The keels, one on each side of the concavity, have forward-pointing hairs and may be prolonged upward into minute "teeth." The entire palea is minutely pubescent, more delicate in texture than the lemma, and is attached to the floret stalk, whereas the lemma is connected to the rachilla. Both lemma and palea adhere to the fruit in maturity.

The margins of the palea "wings" consist of a single layer of lignified cells which appear to be structurally similar to, and continuous with the hypodermal layer. The cell walls of the epidermis are thickest on the rippled external surface. Stomates were noted only in the outer epidermis. Below the hypodermis are one or two layers of thin-walled chlorenchyma, extending to the inner epidermis, which also consists of thin-walled cells.

Each of the structurally similar keels has a vascular bundle, and two to three layers of chlorenchyma (figs. 14, 15). The bundle sheath merges with the epidermis and does not have the endodermis-like layer characteristic of the leaf bundles. Between the keels, the palea is composed of the dorsal and ventral epidermis, with a nonchlorophyllous layer between. The amount of chlorenchyma is greater in the keels than in the margins or in the center.

LODICULES

The two lodicules are inserted on the flower stalk, lie at the basal portion of the inturned "wings" of the palea, and are shorter than the palea (fig. 16). During anthesis, they swell radially, pushing apart the lemma and palea and permitting the anthers to drop out, and the stigma to protrude. Vascularization is present in the lodicules, consisting mostly of xylem elements with annular thickenings.

STAMENS

The brome grass floret has three stamens, one of which is inserted between the two lodicules (fig. 16). The filaments are short at first, but before anthesis they increase several times in length by cell elongation. The filaments are composed mainly of parenchyma with much-elongated nuclei. Two or more annular vessels occur in each filament.

The two-lobed basifixed anther is about 4 mm. long and bifid at both ends. A small mass of tissue, the connectivum, lies between the lobes. The anther wall consists of four layers of cells, epidermis, endothecium, middle layer, and tapetum. The long axes of the epidermal cells are parallel to that of the anther. Stomates are present in the epidermis of the connectivum. The cells of the second or endothecial layer are elongated at right angles to those of the epidermal cells. Thickenings become evident in the endothecium, and this layer is responsible for the dehiscence of the anther. The middle layer has the long axis of its cells parallel with those of the epidermis. The tapetum is the innermost layer; its cells are at first uninucleate and later become binucleate by mitosis and the failure of cell wall formation.

The mature pollen grain is approximately spherical and has a granular exine and a thick intine. There is one germ pore, raised slightly above the surface. Fresh pollen was found to average 37.6 by 42.2μ, and when stained with aceto-carmine consistently shows two sperms and one tube nucleus. The sperms are 8.2 by 4.9μ, and the tube nucleus 13.2 by 8.2μ in diameter. Starch grains are present in the mature pollen grains.

PISTIL

The pistil is borne on a short stalk and consists of the ovary and two styles terminating in stigmatic papillae. The uniloculate ovary has a high, flattened, inner carpel facing the palea, and two distinct, shorter carpels on the side (fig. 16). The outer wall is usually six to eight cells thick, with the inner epidermis chlorophyllous. The base of the ovary contains one large vascular bundle which continues up the high, inner carpel, giving off first a branch to the ovule and then a branch to each style, and terminating before the apex of the ovary is reached.

Each ovary contains a single ovule attached directly to the inner carpel wall, no funiculus being present. The micropyle is directed downward and outward. The ovule is a modified campylotropous type and has two integuments, the outer one disintegrating after fertilization, whereas the inner one finally becomes adherent to the inner ovary wall. Each style arises from one of the outer carpels and divides into two feathery, stigmatic branches. Each branch is composed of four rows of elongated, nucleate cells, the distal ends of which are free and curved outward, thus forming a receptive surface for the pollen. The upper portion of the ovary bears simple hairs above the level at which the lodicules terminate.

ANTHESIS

Panicle primordia are initiated only in the year of anthesis. On May 1, 1941, at Ames, inflorescences were found to be near the ground level and only 1–1.5 cm. long. On May 4 the paleas, lemmas, and glumes were well-developed, and in some cases the lemmas were awned. Stamens and pistils were not in evidence.

Anthesis occurs late in May or early June at Ames, Iowa, although later, sporadic flowering is not uncommon. Anthesis takes place in mid-afternoon. Temperatures influence the rate of anthesis; the process can be hastened by holding mature spikelets in the hand. One floret opens each day in each normal spikelet; the duration of flowering for each spikelet is thus dependent upon the number of florets. Flowering of the panicle begins at or near the top and proceeds basally, but in each spikelet the process proceeds apically.

During the early stages of anthesis, the lodicules become turgid and force the palea and lemma apart. The filaments elongate greatly, and the three golden anthers tip over, usually one to one side and two to the opposite side, between the lemma and palea. The two stigmas "feather" out and one protrudes from each side of the lemma-palea opening. The distal end of the anther splits, and the dry pollen sifts out. On windy days, the anthers were seen to strike the stigmas of their own floret. The palea and lemma spring back somewhat after pollination.

CARYOPSIS

The so-called seed of commerce consists of the caryopsis, palea, lemma, and part of the rachilla. The true fruit or caryopsis is flattened, pointed apically, rounded basally, and has a tuft of hairs at its distal end. The ventral surface has a ridge, and the dorsal surface tends to be slightly concave. The caryopsis varies from 6–8 mm. in length, and measures up to 2 mm. in breadth, and almost 0.5 mm. in thickness.

EMBRYO

The embryo is situated at the base of the dorsal side of the caryopsis, and consists of the scutellum, the coleoptile, one foliage leaf, the stem apex, the radicle, and the coleorhiza. The embryo in soaked seeds is approximately 2 mm. long.

The median scutellar bundle branches at the scutellar plate and supplies the radicle, coleoptile, and shoot apex. The posterior or inner layer of the scutellum, in contact with the endosperm, consists of columnar epithelial cells. Most of the scutellar cells are rich in starch. The single foliage leaf has three large bundles and two smaller ones, the midrib being 90 degrees from each coleoptile bundle (fig. 17).

The radicle is approximately 0.2 mm. in length and tapers slightly toward its flattened distal end, which has a calyptrogen. The radicle is enclosed by the conical coleorhiza, the tissues of which merge with those of the scutellum. White hairs develop upon the coleorhiza soon after it has ruptured the pericarp.

PRIMARY ROOT

Shortly after emergence from the coleorhiza, the primary root has three distinct zones, the epidermis, cortex, and stele. The epidermis and most of the cortex are composed of thin-walled cells, which ultimately disintegrate. The endodermis is a single layer of cells, having greatly thickened and laminated radial and inner tangential walls. Unlignified passage cells occur. The pericycle consists of a single layer of large, radially-elongated cells in the arc between the protoxylem points, and usually two layers of smaller cells elsewhere. There are commonly four of these smaller cells in a group, adjacent to the passage cells of the endodermis.

The vascular tissues have the radial arrangement characteristic of roots. The phloem is arranged in five or six strands, each containing three cells arranged in the shape of a triangle, with the apical cell abutting on the pericycle. This apical cell is four-sided, somewhat diamond-shaped. A phloem strand is derived from a single phloem initial which divides to form two cells, one of which divides again.

Protoxylem vessels are situated between the phloem strands, usually one or two occurring in each xylem arc. The six-sided vessels are smaller than the metaxylem vessels, and are in contact with the small cells of the pericycle. Almost in the exact center of the root there is most commonly one metaxylem vessel, which enlarges before the protoxylem but becomes lignified later (fig. 18).

In the basal portion of a 14-day-old root, all the stelar cells except the phloem were found to be lignified. At 33 days, the protoxylem and metaxylem vessels had developed tyloses, which suggests that the primary root is of little importance as a conductive organ by that time.

ADVENTITIOUS ROOTS

The first adventitious roots arise at the scutellar node, approximately 5 days after germination in the greenhouse. Other roots arise above the first whorl, and by the fortieth day their number and length greatly exceed those of the primary system. The adventitious roots form the permanent root system of the plant. The epidermis gives rise to root hairs a short distance from the tip. The cortex consists of six to nine layers of cells with prominent intercellular spaces. The cells of he cortical parenchyma are elliptical, except those nearest the endodermis, which are somewhat flatter (fig. 3). As the root matures, the cortical cells become flaccid, their walls interlock, and the hypodermis becomes lignified.

In the proximal portion of a 59-day-old adventitious root, the endodermis was found to be well lignified, and no passage cells were evident. The pericycle of the adventitious root, like that of the primary root, is composed of alternate groups of large and small cells. The small cells, opposite the protoxylem points, retain their protoplasm longer than do the large cells.

After the metaxylem vessels enlarge, but before the protoxylem has

become conspicuous, the phloem is recognizable. Each phloem group consists of three cells which develop in the same manner as in the primary root. The phloem strands are in contact with the larger cells of the pericycle and alternate with the protoxylem points. A representative cross-section showed six metaxylem vessels, sixteen phloem groups, and sixteen protoxylem points.

Adventitious roots differ from the primary root in the following details: (1) the adventitious roots are of post-embryonic origin; (2) they have more metaxylem vessels; (3) they have more protoxylem points; (4) they have more phloem groups; (5) the epidermis and cortex function longer; (6) they live more than one year; (7) many are larger in diameter; (8) they have a pith; (9) there are no passage cells in the endodermis.

FIRST INTERNODE

The first internode or "mesocotyl" lies between the scutellar and coleoptilar nodes, and its length increases with depth of planting. The anatomy of the first internode is different from that of the succeeding internodes (fig. 21). The epidermis encloses a broad cortex consisting of thin-walled parenchyma, limited internally by an endodermis and containing one bundle. The pericycle consists of a single layer of hexagonal cells. The vascular system of the stele is composed of four strands; two of the strands are endarch, each with a large group of collateral phloem, the other two strands are exarch, with radial phloem. The limits of this phloem are difficult to recognize. The pith becomes ruptured, forming a central cavity.

COLEOPTILE

The coleoptile contains two bundles, placed 180 degrees apart and each 90 degrees from the scutellar bundle (fig. 17). The two bundles converge toward the apex. Occasionally, the basal portion of the coleoptile has four bundles, but two of these soon terminate. The bundles pass out from the scutellar trace just above the level at which a trace passes into the radicle.

The phloem is on the dorsal side and consists of approximately twenty sieve tubes and companion cells. The xylem usually contains three elements on the ventral side of the bundle, with no clear distinction in size between protoxylem and metaxylem. The remaining coleoptile cells are thin-walled. The coleoptile has an anterior slit approximately 5 mm. from its apex. There is no bud in the axil of the coleoptile.

CULM

Mature culms vary in height from 30–140 cm. The internodes are solid when young but become hollow, the schizogenous cavity being produced by the rupture and collapse of the central parenchyma. The upper internodes are in general longer than the lower, the one below the rachis being the longest. The upper internode is thickest in the middle, whereas the lower internodes are thickest at the apex.

The nodes are composed of a solid diaphragm of parenchyma and numerous vascular bundles. The bundles lie parallel in the internodes but form an interconnecting network in the nodes. The culm itself is not thickened at the nodes.

The structure of the main portion of the culm (fig. 22) is very similar to that of its upper portion, the rachis. The epidermis is heavily lignified on all but its inner walls. The hypodermis is lignified, except for small islands of thin-walled cells which develop chloroplasts in the uncovered parts of the culm. There are two well-defined circles of vascular bundles.

The shoot apex, from which the culm differentiates, is a compact dome of cells, slightly higher than broad. The tunica consists of one or two layers of rectangular cells having dense cytoplasm and large, spherical or elliptical nuclei. Anticlinal cell division predominates. The central core of cells constitutes the corpus, the cells of which are more irregularly polygonal and are characterized by periclinal walls (fig. 23).

SHEATH

The sheath is circular in cross-section and slightly or not at all keeled (fig. 24). It is closed nearly to its summit, but on the side opposite the blade a short slit is present, caused in part by the pressure of the growing culm. Usually the sheath is glabrous, but occasionally those of the lowermost leaves are pubescent. Because of the silica in the outer epidermis, the sheath is scabrous. The base of the sheath is swollen near the node, forming a characteristic thickening at that point. The lower sheaths tend to overlap one another and, unlike the upper sheaths, are often longer than their corresponding internodes.

Stomates are numerous on the dorsal epidermis but are rare or absent on the ventral surface. The latter layer is smooth to the touch and silvery in appearance. On the upper part of the sheath, the dorsal epidermis is most strongly lignified on the outer wall. This lignification occurs after the bundle caps have been lignified. The cells of the dorsal epidermis are somewhat round in cross-section, unlike those of the ventral epidermis. Basally in the sheath, the dorsal epidermis is more heavily lignified, chloroplasts are not as plentiful in the mesophyll, and the ventral epidermal cells are more rectangular. Schizogenous cavities are common between the bundles.

Two types of bundles appear in the sheath. In the larger type, protoxylem is distinguishable from metaxylem. Another feature is a huge bundle cap on the dorsal side (fig. 25). The smaller type of bundle, alternating with the larger and sunken midway into the mesophyll, is encircled by parenchyma, and has only a few xylem elements, a larger number of phloem cells, and only a small bundle cap.

BLADE

The blade was found to vary from 3.5 mm. to 19 mm. in width and from 100 mm. to 400 mm. in length (fig. 2). It is convolute in the bud, and when young, the blades as well as the sheaths are purplish at the base.

Mature blades are flat, taper to a point, diverge at an angle from the stem, are slightly keeled below, almost ridgeless above, and are arranged distichously. The ligule at the junction of the blade and sheath varies in height from 0.5 to 2 mm. and is membranous, short, truncate, and lacerate at its summit. Auricles may be present on the young plants but were not seen on mature specimens.

All young leaves are pubescent on both surfaces, but mature ones usually are glabrous. The edges are scabrous because of the forward-pointing, one-celled, thick-walled spines, 58.8 to 64.7μ long. Each spine has one or two adjacent, short, thick-walled cells averaging about 17.6μ in length.

Stomates are more numerous on the upper surface of the leaf. There are generally two or three rows of stomates, with their long axes parallel with that of the midrib, separated by several rows of long cells. The narrow guard cells have thickenings on their internal faces, and the lumen and nucleus of each is dumbbell shaped. The two accessory cells surrounding the guard cells are wider and have large, elliptical nuclei. The stomates are sunken slightly below the epidermal surface and average 40–41μ in length and 23–24μ in width.

The upper epidermis consists of unspecialized epidermal cells, bulliform cells, stomates, and occasional hairs. The unspecialized epidermal cells lie in rows between the bulliform cells and over the vascular bundles. These cells are slightly narrower over the bundles, becoming progressively larger until they merge with the bulliform cells. In cross-section they are rounded or slightly elliptical, and the outer wall is heavily cuticularized (fig. 26). The large bulliform or "motor" cells occur in three to seven rows, extending the length of the upper surface between the bundles (fig. 26). Reduction of the turgor in the bulliform cells causes their collapse and the consequent rolling of the leaf. The one-celled, nucleate hairs are modified epidermal cells. The lower epidermal cells are of uniform diameter in cross-section, and are externally cuticularized. Stomates and hairs similar to those of the upper epidermis are present. There is no palisade layer. Some of the subepidermal cells are isodiametric, but most of them are irregular, and longitudinal sections show them to be deeply lobed.

Fourteen days after germination, only five bundles may be well developed in a leaf; in a leaf 33 days old, five to seven bundles are usually fully developed; the mature leaf may have twenty-seven or more bundles. The midrib bundle is the largest, and the bundles of the blade are alternately small and large in a definite pattern (fig. 26). The midrib bundle is distinglished by a large wedge-shaped mass of sclerenchyma on the keeled lower side of the midrib, and a large flatter cap on the upper side. The outer walls of the superficial sclerenchyma cells are rippled. A bundle may have sclerenchyma above or below or both, or sclerenchyma may be entirely lacking. The mature fibers in the bundle caps, as determined by maceration, average 11.7μ in width and range from 936 to 1,117μ

in length. Cell walls of the bundle cap become strongly lignified by the fifty-ninth day after germination.

PROPHYLLUM AND TILLER

The buds in the axils of the lower leaves develop tillers and rhizomes and are covered by a protective organ, the prophyllum. The prophyllum has two bundles, 180 degrees apart, like those of the coleoptile. The tillers make their appearance within 8 weeks after germination by breaking through the leaf sheath.

The first emergent leaves of the tillers are atypical in form, the blade being extraordinarily short or absent. This peculiarity has no counterpart in the development of the main shoot. The leaves of the tillers are placed at right angles to those of the main shoot, and the leaves of axillary shoots of a tiller are oriented at right angles to the leaves of the tiller.

RHIZOME

The rhizome is a lateral subterranean organ, aiding in the lateral expansion of the plant and being chiefly responsible for the production of the sodbound condition so characteristic of this species. Certain strains such as Parkland are designated as noncreeping, but they do have short rhizomes. Potted seedlings developed rhizomes about 6 months after germination.

The young rhizomes are white, becoming brown in maturity, and are covered by brown papery scales originating at nodes. The apex of the rhizome develops leaves which expand after the rhizome tip has come to the surface. Nodal buds also arise on the rhizome and subsequently become leafy shoots. The internodes on mature rhizomes averaged 11 mm. in length.

Near the distal end of the rhizome, only the protoxylem elements are lignified, but strong lignification of all xylem elements occurs as the rhizome matures. The tissue sytems of the mature rhizomes are shown diagrammatically in figure 28. Marked lignification is evident in the epipermis, one-layered hypodermis, the endodermis, and pericycle (fig. 29). The endodermis may be locally double.

Vascular bundles lie in contact with and somewhat confluent with the pericycle (fig. 4). A bundle usually consists of the bundle sheath, two to three protoxylem elements, two metaxylem vessels, xylem parenchyma, tracheids, sieve tubes, and companion cells. The lignification of the bundle sheath in the rhizome scales becomes completed before comparable cells in the rhizome have become lignified.

DISCUSSION

The development of the brome grass plant exhibits two phases. The first phase consists of the development of the seedling into a vegetative plant. The shoot apex remains at or near the ground level, but the leaves expand and tillers and rhizomes arise from buds in the axils of the basal

leaves. The adventitious root system assumes dominance over the primary root system, and the plant stores up food reserves.

In the second phase, the internodes elongate rapidly, the inflorescence develops, pollination occurs, and the fruit matures. In the second year, the tillers store up food, develop a root system, and the shoot apex of each tiller elongates rapidly. Shoots develop from the ends of the rhizomes in the first and succeeding years. Bonnett (12) and Evans and Grover (25) have noted much the same events in other grasses.

Comparison of the emergence of organs and anatomical features of brome grass with other grasses reveals some striking similarities and differences. The emergence of the primary root from the side of the coleorhiza in brome grass finds a counterpart in *Holcus sorghum*, as reported by Chi (20). Zinn (77) was of the opinion that this lateral emergence of the primary root is normal for grasses. In *Bromus inermis* the xylem of the primary root develops tyloses as early as the thirty-third day, impairing if not halting water conduction. Percival believed that in *Triticum* the primary root functions up to harvest time (52).

The restriction of the chlorenchyma to the lower half of the glumes, except in the region of the bundles, resembles the condition found in wheat (52). The enclosure of the entire, undifferentiated spikelet by the glumes in smooth brome grass is also characteristic of oats (33). The lemma is inserted on the rachilla and not on the flower stalk and therefore cannot be homologous with a perianth segment. The lodicules, on the other hand, are regarded as a reduced perianth. Hackel (32) homologized the awn of the lemma with the blade of the grass leaf. The part of the lemma above the insertion of the awn is the homologue of the ligule, and that part below is the homologue of the sheath. According to this view, lemmas of brome grass plants that lack an awn, lack the ancestral blade. The spreading of the lemma and palea in anthesis is caused by the swelling of the lodicules as in other grasses. Annular vessels occur in the tissues of the lodicules, a condition similar to that prevailing in *Zea mays* (68) and in other grasses (55).

Smooth brome grass resembles other grasses with respect to the insertion of one of its three stamens between the two lodicules. Stebler and Schröter (60) state that the anthers of grasses are versatile, whereas Bews (11) considers them basifixed. Lindley (44) defines versatile as being attached near the middle, whereby the two halves are nearly equally balanced and swing freely. By this definition, the anthers of smooth brome grass are basifixed. Annular vessels occur in the filament as in wheat and other grasses. The tapetal cells of *Bromus inermis* are binucleate at maturity as in *Agropyron repens* (46) and *Triticum vulgare* (52). Wodehouse (74) characterized the pollen grains of the Gramineae. *Bromus inermis* and *B. erectus* (23) pollen grains each contain two sperms and a tube nucleus, and conform to the general pattern. The sequence of events during anthesis is essentially as described for the species by Beddows (10).

The ovary is interpreted as being tricarpellate, in agreement with the

most widely accepted interpretation of the ovary of the grasses. The innermost layer of the ovary wall appears to be chlorophyllous as in *Poa pratensis* (1), and the vascularization of this organ is simple as Percival (52) found in wheat and Walker (66) found in *Bromus unioloides.*

Boyd and Avery (13), Merry (45), and others have discussed the homologies of the embryo of the grasses. The present writer regards the scutellum as the first leaf and the coleoptile as the second leaf. Both the scutellum and coleoptile are, therefore, greatly modified leaves. The embryo of *Bromus inermis* has no epiblast, a condition said by Bruns (16) to be characteristic of the genus.

Smooth brome grass has only one seminal root, as in timothy (24) and sorghum (19), in contrast with oats (33), which has from one to five. Numerous laterals, originating in the pericycle opposite the phloem, develop subsequently on the primary root as in wheat (52) and sorghum (20). Jeffrey (34) pointed out that the lateral roots of vascular plants originate in the pericycle, opposite the protoxylem points, except in the grasses.

The pericycle consists of both large and small cells. The latter become fully lignified later than the protoxylem, aiding in distinguishing between pericycle and protoxylem. In barley, Hector (33) designates as protoxylem certain cells that seem to occupy the position of the small pericycle cells of *Bromus.*

The phloem is similar in origin and structure to that of sorghum (20). Of the three cells of the protophloem group, the more peripheral cell is a sieve tube, a fact which Chauveaud (18) earlier recognized.

The presence of one or more metaxylem vessels in the center of the primary root is characteristic of grasses, occurring in brome grass, sorghum (20), wheat (52), and rice (33) among others. The expansion of the metaxylem vessels before the maturing of the protoxylem is common in grasses but is unusual for other vascular plants (34). In at least one particular, adventitious roots of brome grass resemble those of *Bouteloua curtipendula* (50), namely, in becoming the important absorptive system by the sixth week after germination.

There has been considerable controversy regarding the homologies of the coleoptile. Sargent and Arber, according to Avery (8), believe that the coleoptile represents two fused stipules, because it commonly has two vascular bundles. However, Percival (52) found two to six bundles in the coleoptile of wheat, and Avery (8) found two to five bundles in that of maize. The latter author (9) believes that this variation in the number of bundles indicates that the coleoptile is homologous with a foliage leaf. The anatomy of the coleoptile bundles in brome grass is very similar to that in wheat (52). Evans (24) prefers to use the term coleophyll (sheath-leaf), rather than coleoptile (sheath-feather).

The border parenchyma, which is a prominent feature of the leaf bundles of brome grass, is said by Lewton-Brain (43) and Carleton (17) to act as a transfusion tissue, delivering food from the mesophyll to the bundle. Directly inside the border parenchyma is an endodermis-like

sheath, also noted by Arber (5) in *Bromus hordeaceus*. The functional relationships of these two sheathing layers are not demonstrable on a purely morphological basis.

Smooth brome grass ligules are without vascularization, as is true of other grasses (5). Philipson (53) concluded that the ligule consists of the free, upper portion of the sheath and a median upgrowth of the adaxial epidermis of the leaf. Kennedy (38) believes the ligule to be a double sheathing axillary stipule. The prophyllum is regarded as a single, modified leaf by Arber (2) and by the present writer.

The histogen theory of Hanstein is not applicable to the shoot apex of smooth brome grass but the tunica-corpus theory of Buder and Schmidt, as restated by Foster (26) seems to be consistent with the observed facts.

Smooth brome grass has well-developed tillers before the eighth week. By way of comparison, Mueller (47) reported that *Bouteloua curtipendula, B. gracilis, Andropogon furcatus,* and *Panicum virgatum* tiller in 3 weeks and *Sorghastrum nutans* tillers in 4 weeks. The internodes on the rhizomes in smooth brome grass average 11 mm. in length whereas the internodes of *Andropogon scoparius* and *Panicum virgatum* are shorter (47). The rhizome scales in smooth brome grass have strong mechanical tissue.

Arber (3, 4) believes amphivasal bundles to be common in the rhizomes of the monocotyledons. Lauder-Thompson (42) found this type of bundle in the nodes of *Spartina townsendii,* and the present work has shown that such bundles occur in *Bromus inermis.*

SUMMARY

A study was made of the gross morphology, anatomy, and development of the smooth brome grass, *Bromus inermis* Leyss.

The radicle breaks out of the side of the white, pubescent coleorhiza. Tyloses developed by the thirty-third day, and the radicle was probably non-functional by that date.

The coleoptile was 10 mm. long by the fifth day, 20 mm. by the fourteenth day, and shredded off by the fifty-fourth day.

The first foliage leaf had emerged by the fifth day; the second leaf was 72 mm. long by the twenty-first day; three more leaves appeared before the fifty-fourth day and as many as ten leaves were present by the one-hundred eleventh day.

Tillers appeared before the fifty-fourth day and had typical leaves, as well as atypical leaves, the blades of the latter being very short or lacking. Rhizomes arose in about six months, emerging from the axils of the lower leaves.

Lateral roots, frequently in pairs, arise from the radicle. The first adventitious roots originate from the scutellar node, but subsequent roots arise from the coleoptilar and higher nodes.

The rachis is anatomically like the culm except that the rachis contains more chlorenchyma. Branches of the rachis contain numerous fiber cells.

A spikelet consists of a rachilla, two glumes, and two to ten florets. Both the first and second glumes have heavily lignified epidermal cell walls, and stomates occur in both the ventral and dorsal epidermis. The arrangement of bundles in the lemma is similar to that of the leaf. The outer walls of the epidermis of the palea are thickened, rippled, and contain stomates. Vascularization is present in the lodicules.

The ovary is tricarpellate and uniloculate. One vascular strand extends into the inner lobe, and branches into the ovule and into each style.

A mature pollen grain contains a tube nucleus, two sperms, and numerous starch grains.

The structure of the embryo conforms to the structure of the typical gramineous embryo, having a prominent scutellum, a radicle encased in a coleorhiza and a coleoptile enclosing the shoot apex. Only one foliage leaf primordium is present. No bud was found in the axil of the coleoptile.

The anatomy of the roots, first internode, culm, leaves, and rhizomes is also described in detail. No marked divergence from the characteristic pattern of the Gramineae was found.

PLATE I

Fig. 1. Panicles of two selections of *Bromus inermis*, approximately ¼ natural size.
2. Variation in width of blade of miscellaneous selections, approximately ¼ natural size.
3. Cross-section of the adventitious root. × 138.
4. Detail of a section of the rhizome. × 300.

PLATE I

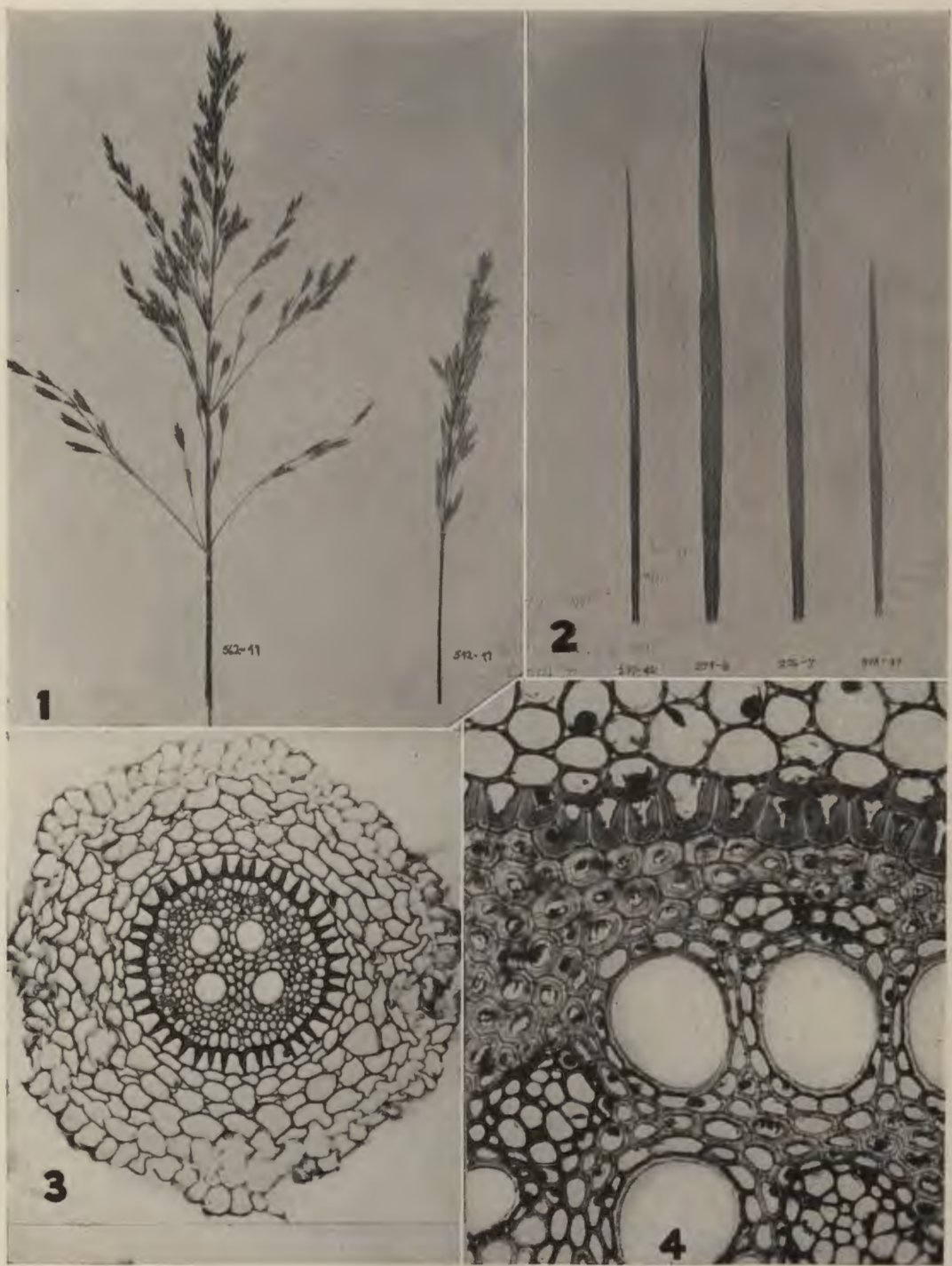

PLATE II

5. Detail of a section of the rachis. × 233.
6. Cross-section of a secondary branch of the rachis. × 133.
7. Outline of the second glume showing three vascular bundles. × 67.
8. Detail of a section of the second glume. × 300.
9. Detail of the first glume. × 233.
10. Outline of the first glume showing one bundle. × 100.
11. Detail of the lateral tip of the first glume. × 233.

PLATE II

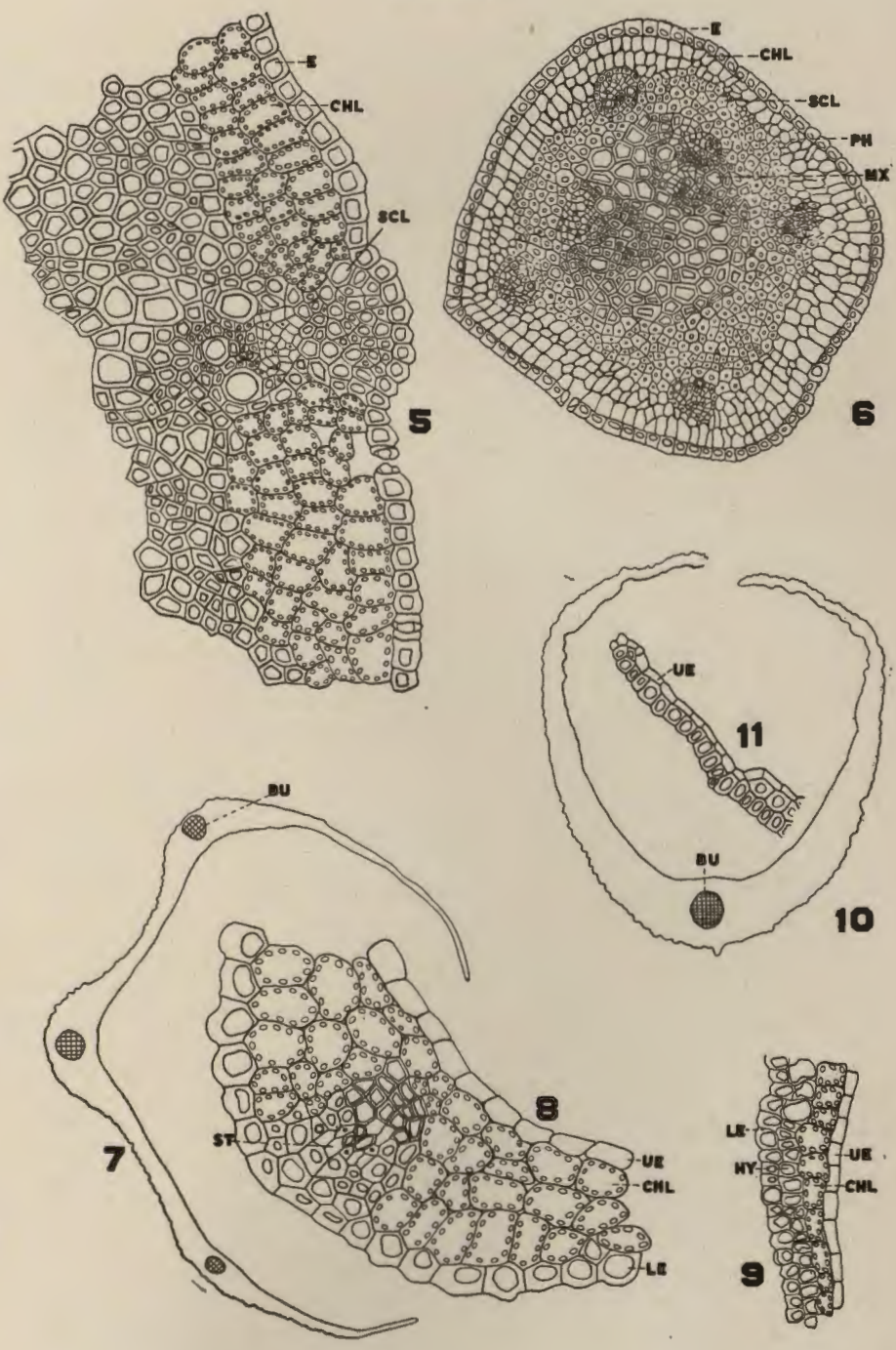
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PLATE III

12. Outline of the lemma showing seven bundles. × 67.
13. Detail of a section of the lemma. × 233.
14. Outline of the palea showing a bundle in each keel. × 67.
15. Detail of a palea keel. × 300.
16. Cross-section of the ovary, lodicules, and filaments. × 67.
17. Cross-section of the embryo through the plumule, showing the position of vascular bundles in the scutellum, coleoptile, and leaf. × 100.

PLATE III

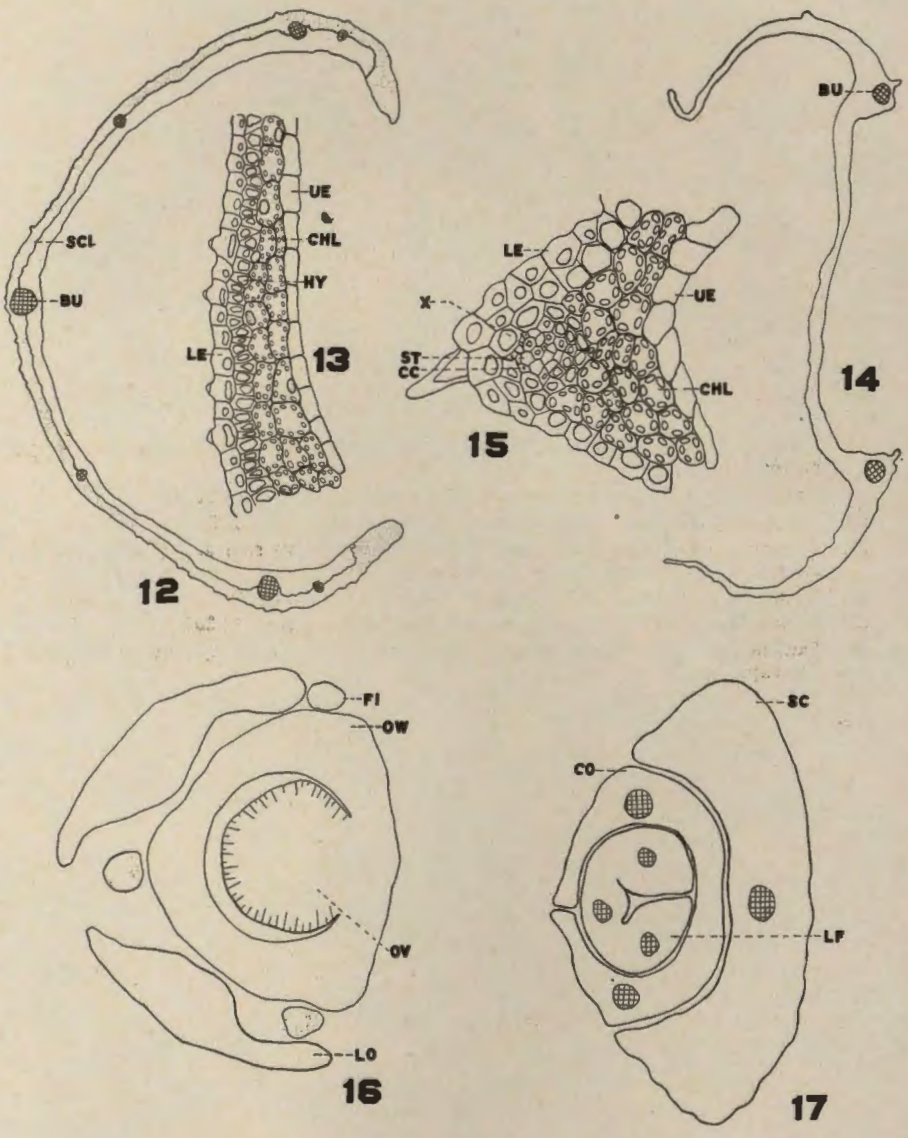
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PLATE IV

18. Cross-section of a mature primary root showing the disintegration of the cortex. × 200.
19. Cross-section of the stele of a young adventitious root. × 100.
20. Cross-section of the stele of an old adventitious root. × 200.
21. Cross-section of the first internode showing relative size and position of the stele and the cortical bundle. × 67.
22. Detail of a section of the culm. × 133.
23. Longitudinal section of the growing point of the stem. × 200.
24. Outline of a leaf sheath showing the extraordinary development of the bundle caps. × 27.

PLATE IV

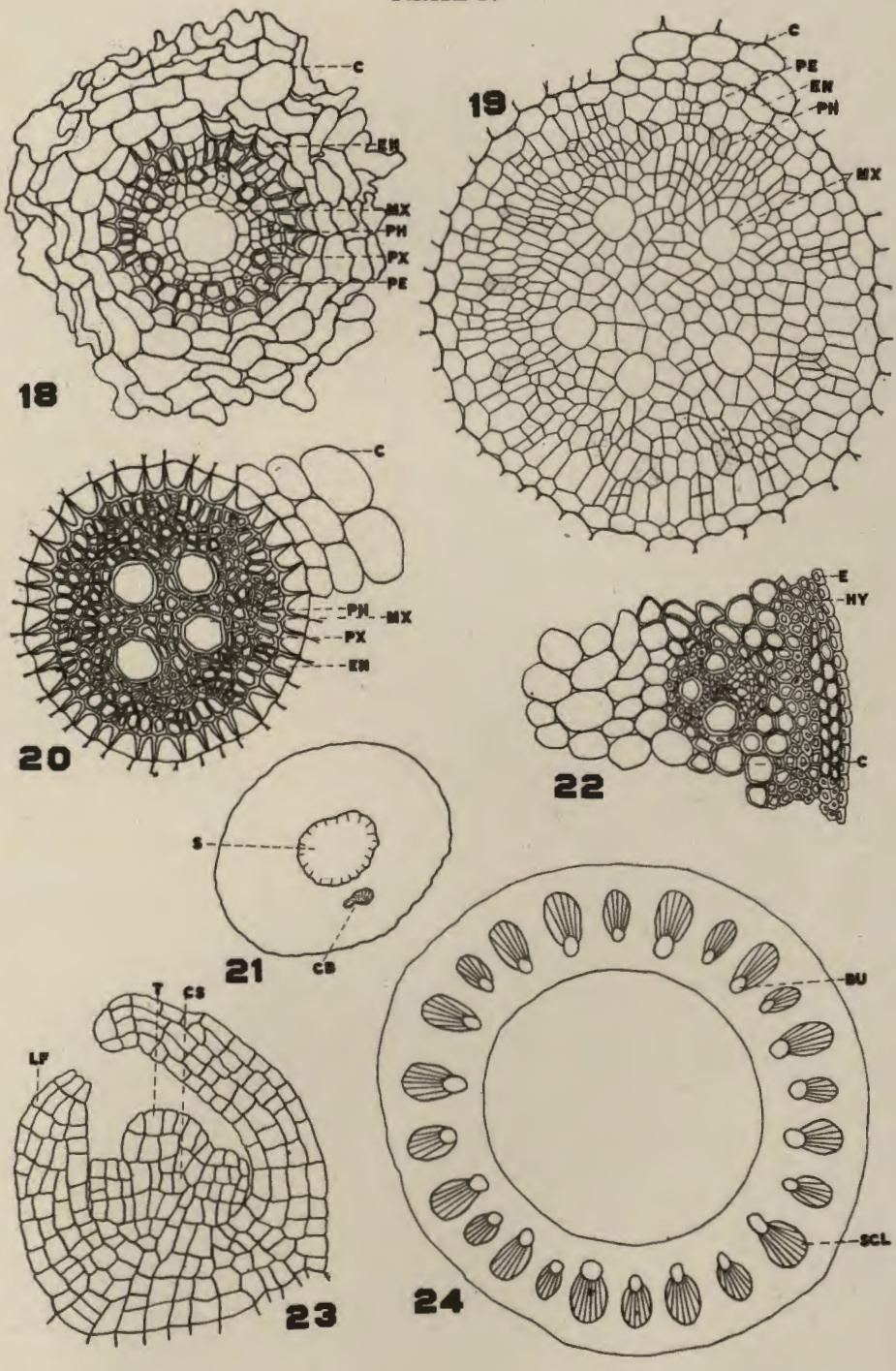
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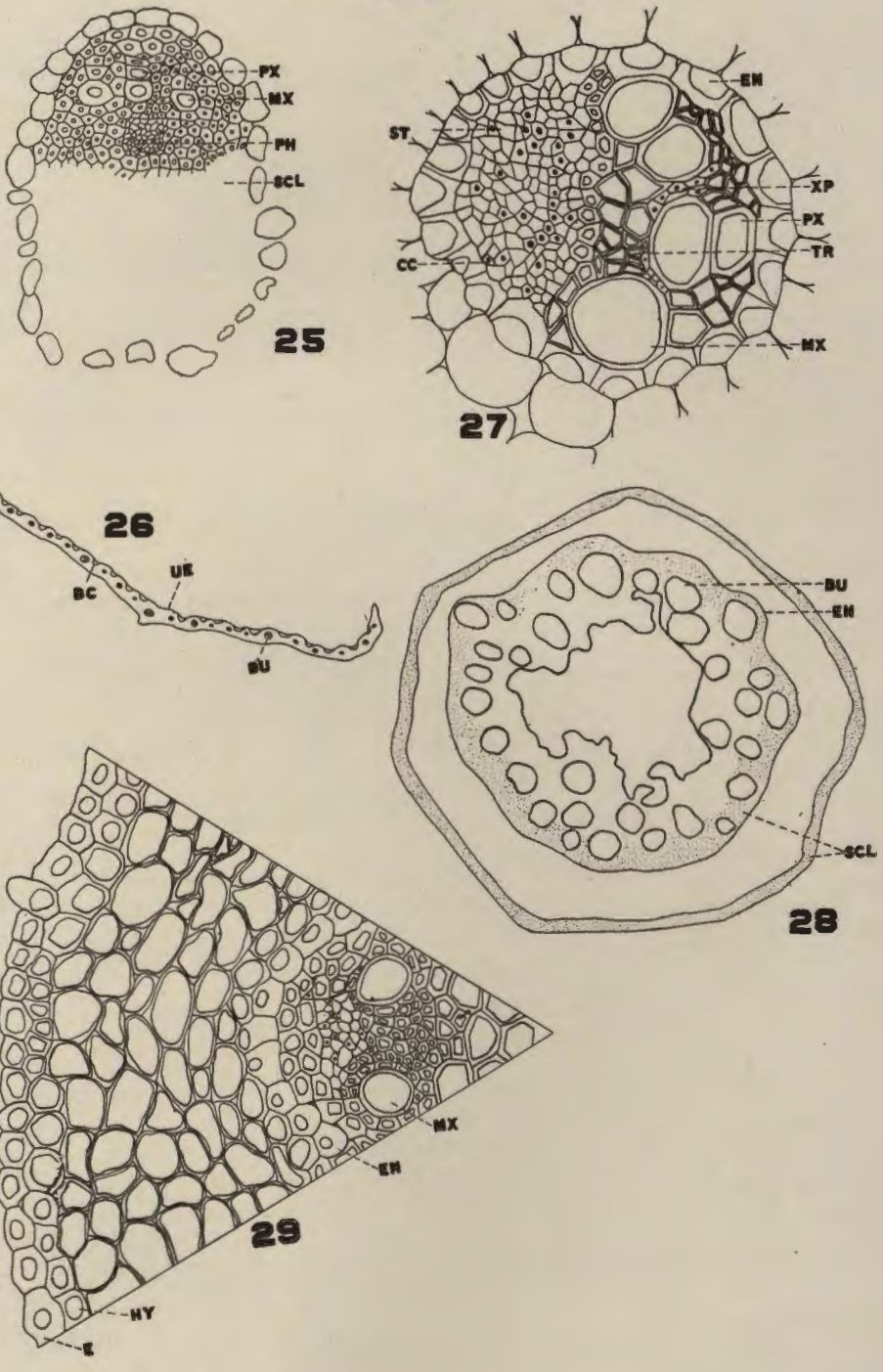
PLATE V

25. Detail of a sheath bundle, with most of the detail of the sclerenchyma omitted. × 133.
26. Outline of a leaf showing the relative position of the bundles and bulliform cells. × 14.
27. Detail of a leaf bundle. × 600.
28. Outline drawing of an old rhizome showing the position of the bundles. × 50.
29. Detail of a section of an old rhizome. × 233.

EXPLANATION OF ILLUSTRATIONS

BC—bulliform cells
BU—Vascular bundle
C—cortex
CB—cortical bundle
CC—companion cell
CHL—chlorenchyma
CO—coleoptile
CS—corpus
E—epidermis
EN—endodermis
FI—filament
HY—hypodermis
LE—lower epidermis
LF—leaf
LO—lodicule
MX—metaxylem
OV—ovule
OW—ovary wall
PE—pericycle
PH—phloem
PX—protoxylem
S—stele
ST—sieve tube
SC—scutellum
SCL—sclerenchyma
T—tunica
TR—tracheid
UE—upper epidermis
X—xylem
XP—xylem parenchyma

PLATE V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CONCERNING AMERICAN RHOPALINI (HEMIPTERA, RHOPALIDAE)

H. M. HARRIS

From the Department of Zoology and Entomology, Iowa State College

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For several years I have been concerned in spare moments with a study of certain genera of the hemipterous family Rhopalidae. Originally it was the intention to prepare monographic treatises of the different groups, but it early became apparent that for many reasons such could not be done and that the only practicable procedure was to issue reports of the studies from time to time. The present paper, the seventh in the series, is in continuation of these reports. It reviews the American species of Stictopleurus Stål and gives notes and illustrations[1] to aid in the recognition of the American genera of Rhopalini.

THE SPECIES OF STICTOPLEURUS

The genus Stictopleurus Stål is set apart from other genera of Rhopalini by reason of the characters of the metapleuron, pronotum, antennae, and genitalia. The body form, the vestiture, and the nature of the scutellum also are somewhat characteristic. The name Stictopleurus (= punctured pleuron) is descriptive of one of the distinctive features. The group is largely boreal and holarctic in distribution. As in other genera of the family the species vary much in color, size, and structural features.

There is a distinct need for a careful revisionary study in which particular attention is given to the genitalic characters. The type specimen of each described species must be re-examined. Data on the biology and ecology of some forms will need to be assembled and analyzed. Only by such an inclusive study can the true relationship of the American species to one-another and to those that occur in Asia and Europe be known. The following notes on the American forms will aid the investigator who is in a position to attempt such a study, and in the meantime, will dispose of some confusing points regarding synonymy and serve to aid workers to identify American specimens.

GENERIC CHARACTERS

Any attempt to redefine the genus without more exact knowledge of a greater number of extra-American species than I now possess must necessarily be of limited worth. However, an enumeration of those general features which together appear to set apart the species complex from other groups of species is offered below:

Form oblong, somewhat depressed; body not distinctly setose, the clothing hairs mostly in the nature of fine, soft, short, recumbent pubes-

[1] I am indebted to Mr. George Hopping for the figures in Plate I and to Mr. Judson McGuire and Dr. Reid Davis for those comprising Plate II.

cence. Punctures, especially of pronotum, coarse, close together, irregular, the interspaces somewhat rugulose. Antennal I surpassing head by approximately half its own length. Jugum narrow, produced anteriorly slightly beyond the truncate front end of lorum. Antennal shelf prominent, its apex forming a distinct antenniferous tubercule. Bucculae rather sharply declivent. First rostral segment surpassing bucculae by about half its own length. Pronotum (fig. 20) with front angles slightly swollen and somewhat prominent; the cicatrices on front lobe ending on each side in a more or less complete loop that surrounds or encloses a raised island; the collar punctate, separated from cicatrices by a smooth transverse ridge. Metapleuron not sharply divided into two parts, of about the same texture throughout, rather regularly coarsely punctate, the hind margin almqst truncate and nearly perpendicular to the upper margin (fig. 9). Osteoles obsolete, the canals absent. Scutellum small, the base shorter than the sides, the latter sinuate, constricted distinctly behind the middle.

The male genitalia (figs. 1–4) show excellent specific characters, and also generic distinctness in that the general nature of the capsule and claspers is markedly different from that prevailing in other genera. In the past it has been extremely difficult and often impossible to establish the specific identity of female examples, but it now appears that the female genitalia offer worthwhile recognition features (figs. 15–18).

The type species of the genus (logotype, designatd by Oshanin in 1912) is *Stictopleurus crassicornis* (Linn.).

KEY TO SPECIES

1. Male 2
   Female 6
2. Genital clasper with a pronounced globose enlargement basally, strongly tapering distally, recurved so that the posterior face is concave (fig. 4) ..... *punctiventris* (Dallas)
   Clasper, without sub-basal enlargement, flat or curved anteriorly 3
3. Clasper flat, tapering (fig. 1). General body color usually of a pronounced reddish hue ..... *knighti* Harris
   Clasper curved anteriorly, the posterior surface convex. General color usually decidedly greenish 4
4. Clasper somewhat expanded and spoon-shape distally (fig. 2) ..... *plutonius* (Baker)
   Distal part of clasper straplike, the sides more nearly parallel, the apex prominently, angularly emarginate 5
5. Size small (5.0–5.6 mm.). Clasper narrower. Upper lateral edge of genital capsule not produced (fig. 3) ..... *viridicatus* (Uhler)
   Size larger (6.6–7.1 mm.). Clasper broader. Dorso-lateral edges of capsule somewhat produced ..... *intermedia* (Baker)
6. Size large for the group (length, 7.2–8.6 mm.; width of abdomen, 3.1–3.6 mm.). Antennal I usually somewhat stouter than IV and only about twice as long as thick 7
   Size small (length 5.6–7.1 mm.; abdominal width, 2.2–2.7 mm.). Antennal I hardly as stout as IV and usually about 2½ times as long as thick 9
7. Opening at apex of venter exposing genital region, as viewed from the rear, broad, fully as wide as or wider than deep 8
   Opening exposing genital region not or scarcely broader than deep. Upper edge of urite IX not or slightly produced. Valves of ovipositer and urite beset with short spinules (fig. 15) ..... *intermedia* (Baker)
8. Body stouter. Pronotum rather strongly declivent. Color not distinctly reddish, usually grayish-yellow with dark markings. Opening at apex of venter (fig. 16) more broadly U-shaped ..... *punctiventris* (Dallas)

Body more depressed, the pronotum flatter. Color distinctly reddish. Opening at apex of venter, as seen from the rear, more distinctly V-shaped .... *knighti* Harris

9. Urites produced and sub-contiguous above the valves (fig. 17). Color often darker, the membrane then with fuscous streaks. Collar more sharply marked off. Vertex less arched. Pronotal punctures somewhat finer, less rugulose. Rostral I slightly shorter. ..... *plutonius* (Baker)

Urites less strongly produced dorsally (fig. 18). Color usually more or less clear greenish yellow, with less pronounced dark markings. Collar less clearly delimited. Vertex more distinctly arched in front of ocelli. Pronotal punctures slightly more profound and rostral I faintly longer..... *viridicatus* (Uhler)

Stictopleurus punctiventris (Dallas)

1852 *Rhopalus punctiventris* Dallas, List of Hemip., 2:526.
1859 *Corizus novaeboracensis* Signoret, Ann. Soc. Ent. Fr., (3) 7:97.
1861 *Corizus borealis* Uhler, Proc. Acad. Nat. Sci. Phila., 12:284.
1872 *Corizus borealis* Uhler, Hayden's Survey Terr., Rept. for 1871, p. 403.
1876 *Corizus punctiventris* Uhler, Bull. U. S. Geol. Geog. Surv., 1:301.
1878 *Corizus punctiventris* Uhler, Bull. U. S. Geol. Geog. Surv., 4:505.
1885 *Corizus punctiventris* Provancher, Pet. Faune Ent. Can., 3:60.
1889 *Corizus punctiventris* Van Duzee, Can. Ent. 21:2.
1893 *Corizus puntiventris* Uhler, Proc. Ent. Soc. Wash., 2:370.
1894 *Corizus punctiventris* Uhler, Proc. Calif. Acad. Sci., (2) 4:237.
1895 *Corizus punctiventris* Gillette and Baker, Hemip. Colo., p. 21.
1904 *Corizus novaeboracensis* Van Duzee, 20th Rept. N. Y. St. Ent., p. 549.
1906 *Corizus punctiventris* Barber, Brooklyn Inst. Sci. Bul., 1:272.
1908 *Corizus crassicornis* Van Duzee, Can. Ent., 40:110.
1908 *Corizus crassicornis* Hambleton, Ann. Ent. Soc. Amer., 1:137, Pls. VIII & IX.
1908 *Corizus novaeboracensis* Baker, Can. Ent., 40:242.
1908 *Corizus novaeboracensis occidentalis* Baker, Can. Ent., 40.243.
1917 *Corizus crassicornis* Van Duzee, Cat. Hemip., p. 123.
1919 *Corizus crassicornis* Gibson, Can. Ent., 51:89.
1923 *Corizus crassicornis* Parshley, Hemip. Conn., p. 752
1928 *Corizus crassicornis* Blatchley, Heterop. E. N. Amer., p. 277.
1937 *Corizus punctiventris* Harris, Iowa St. Coll. Jour. Sci., 11:172.
1941 *Corizus crassicornis* Torre-Bueno, Ann. Ent. Soc. Amer., 34:285.
1941 *Corizus crassicornis* Torre-Bueno, Ent. America, 21 (NS):93.
1943 *Stictopleurus punctiventris* Harris, Iowa St. Coll. Jour. Sci., 17:203.

S. punctiventris varies much in size, general color, and markings. The descriptions by Hambleton (1908) and Blatchley (1928) are in general accurate and detailed, and are recent enough to preclude the need for a redescription here. Accurate determination is contingent upon a careful study of the genitalia. The male claspers (figs. 4 and 19) are distinctive. In the female the tip of the venter is more rounded as seen from the side (fig. 13) and is slightly emarginate at the mid-ventral line. The apical orifice exposing the genitalia is distinctly transverse, and the valves are proportionally shorter (fig. 16) than in related species.

SIZE: Length, male 6.5–7.0 mm.; female, 7.2–7.9 mm. Width, pronotum, 2.0–2.7 mm.

RANGE: *S. punctiventris* (Dallas) ranges across Canada and the northern part of the United States and southward along the mountain chains. I have examined specimens from the following states and provinces: Alberta, British Columbia, California, Colorado, Idaho, Indiana, Maine, Michigan, Minnesota, Montana, Nevada, New Hampshire, New York, Nova Scotia, Ohio, Ontario, Oregon, Pennsylvania, South Dakota, Utah, and Washington. The species is recorded in the literature, under

the names *crassicornis* and *borealis*, from Arizona, Massachusetts, Mexico, Nebraska, and New Mexico. Essentially nothing is known regarding its biology.

Comparison of the genitalia of specimens from the United States with specimens from Europe, and with Ribaut's excellent figures of the European species, several years ago disclosed that this widespread American form is not conspecific with *crassicornis* (L.), as had been held to be the case and led to the resuscitation for it of the name *punctiventris* Dallas. It is the commonest and most widely distributed member of the genus in America and had been described under several names. Uhler (1872) seems to have been the one first to suggest that his *Corizus borealis* might be synonymous with *Rhopalus punctiventris* Dallas. He noted, also, that it was close to *crassicornis* (Linn.) and called attention to the great variation in color within the species. Later (1876) Uhler synonymized *borealis* with *punctiventris*. I have seen the type of *borealis* (Uhl.), a male, from S. Colorado, in the collection of the U. S. National Museum, and can verify that it is the species here treated as *S. punctiventris*. Dallas' type of *punctiventris* presumably is in the British Museum of Natural History and has not been available for this study.

On the authority of Horvath, Van Duzee (1908) synonymized Signoret's *novaeboracensis* with *crassicornis* (L.), and Hambleton (1908) identified *novaeboracensis* with *punctiventris*, both of which names he considered as synonyms of *crassicornis* (Linn.).

Baker (1908) in his treatment of *novaeboracensis*, proposed several new names for what he called "the commoner forms of this species," Although he really gave no description, his key of less than a dozen lines appears to be sufficient to establish the names used. A few years back I made a search of the U. S. National Museum for Baker's specimens and, thanks to the splendid cooperation and expert help of Mr. Harry G. Barber am able to dispose of these names as follows:

Corizus novaeboracensis pallidus Baker. This name must stand as a synonym of *Stictopleurus viridicatus* (Uhler) and is discussed on a later page.

Corizus novaeboracensis intermedią Baker appears specifically distinct and is treated below as *Stictopleurus intermedia* (Baker).

Corizus novaeboracensis plutonius Baker is dealt with later as *Stictopleurus plutonius* (Baker).

Corizus novaeboracensis novaeboracensis Baker and *Corizus novaeboracensis occidentalis*, as represented by specimens from Colorado, in the U. S. National Museum and bearing Baker's determination labels, are outright synonyms of *Stictopleurus punctiventris* (Dallas).

Stictopleurus knighti Harris

1942 *Stictopleurus knighti* Harris, Jour. Kan. Ent. Soc., 15:100.

This splendid species is easily recognized by the genitalic characters (fig. 1). In general it is more roseate than *punctiventris* and has a

slightly different facies. The original description is sufficiently recent that it need not be repeated here.

SIZE: Length, male, 6.6–7.0 mm.; female, 7.6–7.9 mm. Width, pronotum 2.08–2.38 mm.

RANGE: Heretofore the species has been known only from Minnesota, but I now can record a female specimen from Thompson, Michigan, August, 1937, and a male, Agricultural College, Michigan, April 28, 1892, P. R. Uhler collection. The right paramere of this latter individual is atypical, apparently having been injured in development.

Stictopleurus viridicatus (Uhler)

1872 *Corizus viridicatus* Uhler, Hayden's Survey Terr., Report for 1871, p. 404.
1876 *Corizus hyalinus* Uhler, Bull. U. S. Geol. Geog. Surv., 5 (2nd Series):300 (in part).
1877 *Corizus hyalinus* Uhler, Bull. U. S. Geol. Geog. Survey, 3:407.
1908 *Corizus viridicatus* Horvath, Ann. Mus. Natl. Hung., 6:556.
1908 *Corizus viridicatus* Hambleton, Ann. Ent. Soc. Amer., 1:138.
1908 *Corizus novaeboracensis pallidus* Baker, Can. Ent., 40:243.
1914 *Corizus viridicatus* Barber, Jour. N. Y. Ent. Soc., 22:171.
1919 *Corizus viridicatus* Gibson, Can Ent., 40:89.
1928 *Corizus viridicatus* Blatchley, Heterop. E. N. Amer., p. 277.
1941 *Corizus viridicatus* Torre-Bueno, Ann. Ent. Soc. Amer., 34:285.
1941 *Corizus viridicatus* Torre-Bueno, Ent. Amer., 21(NS):94.

This species was described from specimens collected in Colorado, Nebraska, and Dakota. A careful analysis of the original description leads one to believe that the type series must have included specimens of the species *viridicatus* and also pale individuals of the older *Liorhyssus hyalinus* (Fabr.). This suspicion is intensified by the fact that Uhler himself, in 1876, discarded the name *viridicatus* in favor of *hyalinus* and the following year spoke of *viridicatus* as a variety of *hyalinus*. Certain features set forth in Uhler's description, however, make it clear that he had before him some individuals of the species now recognized as *viridicatus*, e.g.: "front of face rather blunt. Apical joint of antennae rather slender, hardly longer than preceding. Posterior flap of metapleura oblique truncated, with the upper angles rounded at tip, and together with acetabula caps minutely punctured. Lateral edge of scutellum recurved, the tip sunken, and its apex almost acute." Hambleton (1908) and Horvath (1908), working separately, established the fact that *viridicatus* Uhler is specifically distinct from *hyalinus* (Fabr.). These workers correctly called attention to the kinship between *viridicatus* and *punctiventris* (Dallas), and Horvath pointed out that *hyalinus* is referable to Liorhyssus while *viridicatus* is a true Stictopleurus. In the U. S. National Museum collection is a female individual from Colorado, labelled in Uhler's handwriting "*Corizus viridicatus* Uhler, Type." In my opinion this individual is the female of the species here recognized. Also in the museum there are specimens bearing Baker's identification labels "*C. novaeboracensis* var. *viridicatus*." These latter unquestionably represent the "smaller, pale greenish, western form" for which Baker proposed the name *Corizus novaeboracensis pallidus*. There appears to be in the

museum no specimen bearing Baker's label "pallidus," a name which is preoccupied in the genus by Sahlberg's 1878 usage for a Siberian species.

As in other species of the genus, *viridicatus* varies much in size and in the amount and intensity of dark markings. For the general picture one may refer to the descriptions by Uhler, Hambleton, and Blatchley. The species is distinctly smaller than *S. punctiventris* (Dallas). The vertex is not so flat, the pronotum has less distinct impressions within the humeri and finer, more even punctations, the basal and apical antennal segments are less incrassate, and the basal rostral segment is proportionately slightly longer. The male clasper and genital segment are characteristic (fig. 3). In the female the shape of the apical abdominal segment, as seen from the side and from beneath, and the characters of the enclosed genital segments (fig. 18) are noticeably different from those of *S. punctiventris*. The species is very close to *S. plutonius* and separable from it only by close study.

SIZE: Length, male, 5.1–5.6 mm.; female, 5.6–6.8 mm. Width, pronotum, 2.0–2.3 mm.

RANGE: I have before me specimens of *S. viridicatus* from Alberta, Arizona, Colorado, Idaho, Iowa, Kansas, Minnesota, Montana, Nebraska, New Mexico, North Dakota, Saskatchewan, South Dakota, Utah, Washington, and Wyoming. The species has been recorded in the literature from California and District of Columbia. The latter record, based on a specimen in the Heidmann collection, appears extralimital and needs confirmation.

S. viridicatus is very close to *S. nysioides* (Reuter) from Siberia as represented by two female examples kindly sent me some years ago by Dr. A. N. Kiritshenko. A careful comparison of the male genital characters is needed to make clear the relation between these two species.

Stictopleurus plutonius (Baker)

1908 *Corizus novaeboracensis plutonius* Baker, Can. Ent., 40:243.
1944 *Stictopleurus plutonius* Harris and Shull, Ia. St. Coll. Jour. Sci., 18:202.

Closely related to *S. viridicatus* (Uhler) and heretofore confused with it and with *S. punctiventris* (Dallas), but recognizable by the genital characters.

Size, form, and vestiture about as in *viridicatus* (Uhler), punctation perhaps a bit coarser and more rugose. Color variable as in *viridicatus*, but often more conspicuously marked with black. Head noticeably declivent in front, the vertex and frons more definitely rounded above than in *punctiventris*, but less so than in *viridicatus*. Antennae with segment I greatly surpassing tylus, only slightly swollen, not stouter than IV; proportion of segments 11 : 22 : 22 : 25. Antenniferous tubercules from above slightly shorter and more obtuse than in *viridicatus*. Bucculae scarcely enclosing basal half of first rostral segment. Rostrum attaining metasternum; segment I just reaching prosternum, faintly shorter than in *viridicatus* (Uhler). Male with upper edge of genital segment more angularly produced than in *viridicatus*, the clasper larger, its apex broader and

somewhat spoon-shaped with the convex side to the rear (fig. 2). Female genitalia narrow as in *viridicatus,* the valves higher, the upper angle of the ninth urites produced and subcontiguous above the valves (fig. 17).

SIZE: Length, male, 5.0–5.5 mm.; female, 5.6–7.1 mm. Width, pronotum, 2.4–2.9 mm.

RANGE: I have seen examples from Colorado, Idaho, Nevada, Oregon, Utah, Washington, and Wyoming.

There are in the National Museum collections, apparently, no specimens of *plutonius* determined as such by Baker. However, there is a male from Colorado bearing Baker's label, "*Corizus novaeboracensis* var. *niger* Baker." It becomes obvious when one studies this specimen and others of the larger, more melanistic examples of this species that it must represent his *plutonius,* and that for some reason the manuscript name *niger* was discarded in favor of *plutonius.* The species is very closely related to *viridicatus.* In general it is more northern in distribution, and to judge from the many examples I have seen, is the more abundant of the two forms in Idaho and Washington, while *viridicatus* is dominant in Colorado and New Mexico.

Host plant records and life-history notes will be necessary to reveal the true relationship of this form to *viridicatus.*

Stictopleurus intermedia (Baker)

1908 *Corizus novaeboracensis intermedia* Baker, Can. Ent., 40:243.

The name *intermedia* was given by Baker to a series of specimens from Ormsby Co., Nevada, collected in July, and set apart because of their pale brown color and yellow scutellum. Examples from this series of individuals are present in the University of Kansas Snow Collection and in the U. S. National Museum collection, some of the latter bearing Baker's determination labels. Heretofore, the form has been considered as no more than a color variation of *punctiventris* (Dallas), but study of the genitalia discloses that there are rather constant differences in both male and female individuals. In many ways the form appears intermediate between *punctiventris* and *viridicatus,* and it will remain for the future to disclose the true relationship of these forms.

Size and shape about as in *punctiventris,* the body perhaps slightly more nearly parallel-sided. Color variable as in the other species, in pale examples the scutellum and connexivum often immaculate, melanistic examples with strongly maculate connexivum not uncommon, however. Pronotum with median line and lateral edges paler. Male genital segment somewhat as in *punctiventris,* the clasper more nearly like that of *viridicatus.* Female venter narrower (more laterally compressed) at the apex than in *punctiventris,* genital segments with the urites much less strongly produced and distinctly spinulose (fig. 15).

SIZE: Length, male, 6.7–7.1 mm.; female, 7.5–8.1 mm. Width, pronotum, 2.0–2.6 mm.

RANGE: Forty-four specimens are at hand from Colorado, Montana, Nevada, Oregon, Utah, and Washington.

MISCELLANEOUS NOTES ON RHOPALINI

Torre-Bueno (Ann. Ent. Soc. Amer., 34:284-288, 1941) has contributed a splendid word picture of the state the taxonomist finds himself in when attempting to deal with members of this group. Harris reviewed the history of the family name (Jour. Kan. Ent. Soc., 15:63-64, 1942) and presented keys for the separation of the tribes and genera together with comments on their synonymy and validity (Ia. St. Coll. Jour. Sci., 17:197-204, 1943). The following notes and the illustrations are presented as further contributions toward a clarification of the relationships between these groups.

Liorhyssus Stål: The male genitalia and the female abdominal segments in the species of Liorhyssus known to me are of the same general nature, but show specific differences. The type of male genitalia is sufficiently different from that found in other groups of species of Rhopalini as to lend strong support for the thesis that Liorhyssus should be accorded generic status. Figure 8 portrays the genital capsule of *L. hyalinus* (F.) as viewed from the rear; while figures 12 and 21 show the key characters of the metapleuron and pronotum respectively.

Niesthrea Spinola: As in Stictopleurus and Liorhyssus the genital characters in Niesthrea show specific differences but are of the same generic type. This is evident from a comparison of the genital capsules of *N. sidae* (Fabr.) (fig. 7) and *N. pictipes* Stål. (fig. 6) with those of species of other genera. *N. pictipes* (Stål) clearly deserves specific identity and is here resurrected from synonymy. Numerous other valid species, many now sunken in synonymy, occur in the tropics.

Arhyssus Stål: The American species of Arhyssus appear to fall into two groups centered around *A. bohemanii* (Sign.) and *A. scutatus* Stål, respectively. Elsewhere I have shown that the *scutatus* complex is composed of several distinct species (Jour. Kan. Ent. Soc. 15:100-105, 1942). Figures 10 and 11 show the metapleura of *A. barberi* Harris and *A. parvicornis* (Sign.), respectively. Figure 22 pictures the pronotal characters of *A. barberi;* and the genital structures of *A. lateralis* (Say) are portrayed in figure 5.

PLATE I. MALE GENITAL CAPSULES

1. *Stictopleurus knighti* Harris.
2. *Stictopleurus plutonius* (Baker).
3. *Stictopleurus viridicatus* (Uhler)
4. *Stictopleurus punctiventris* (Dallas).
5. *Arhyssus lateralis* (Say).
6. *Niesthrea pictipes* (Stål).
7. *Niesthrea sidae* (Fabr.).
8. *Liorhyssus hyalinus* (Fabr.).

PLATE I

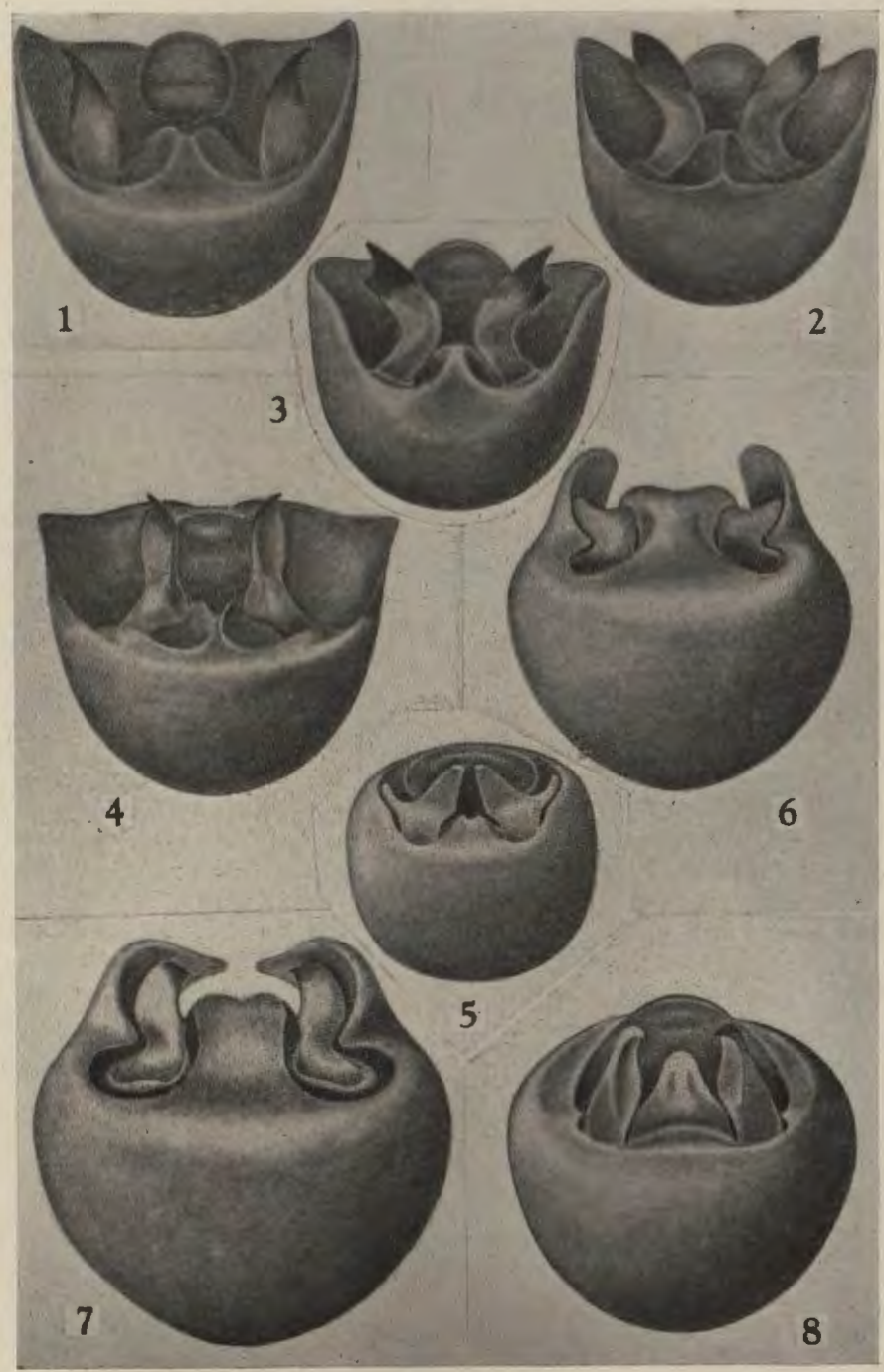
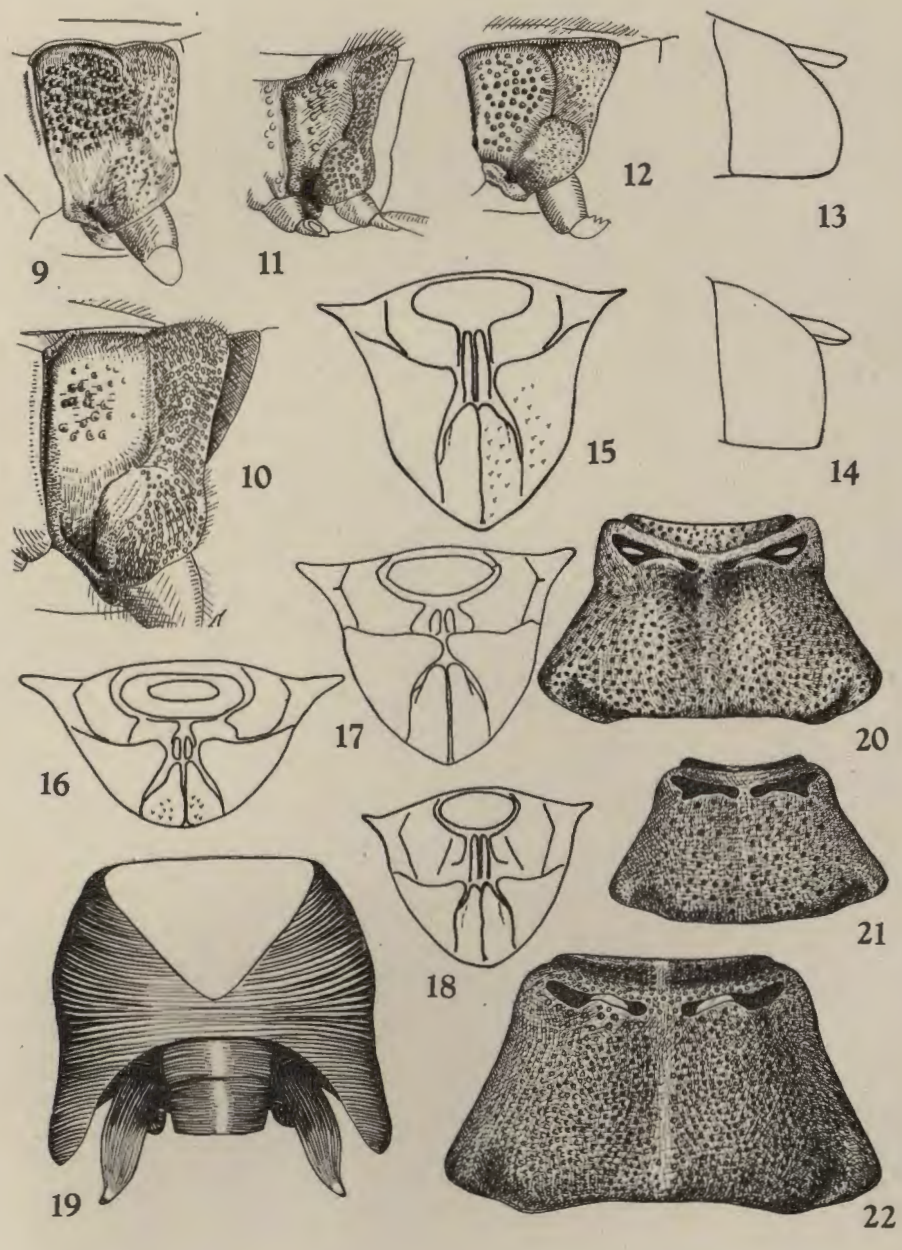


PLATE II. KEY CHARACTERS OF AMERICAN RHOPALINI

9. Metapleuron of *Stictopleurus punctiventris* (Dallas).
10. Metapleuron of *Arhyssus barberi* Harris.
11. Metapleuron of *Arhyssus parvicornis* (Sign.).
12. Metapleuron of *Liorhyssus hyalinus* (Fabr.).
13. Tip to venter, lateral aspect, of female *S. punctiventris*.
14. Tip of venter, lateral aspect, of female *S. plutonius*.
15. Female genital segments, caudal aspect, *S. intermedia* (Baker).
16. Female genital segments, *S. punctiventris* (Dallas).
17. Female genital segments, *S. plutonius* (Baker).
18. Female genital segments, *S. viridicatus* (Uhler).
19. Male genital capsule, dorsal aspect, *S. punctiventris*.
20. Pronotum, *S. punctiventris* (Dallas).
21. Pronotum, *Liorhysus hyalinus* (Fabr.).
22. Pronotum, *Arhyssus barberi* Harris.

PLATE II



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